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РЕЗЮМЕ

СИНТЕЗ КУКУРУЗЫ С ДВУХРЯДНЫМ ПОЧАТКОМ

Л. ДАНИЕЛ

Инцухтируя материал **Hepperly** (1949) с нечетным числом рядов зерен, потом повторно скрещивая с четырехрядными линиями, удалось синтезировать кукурузу со стоящими по одному колосками и двухрядными початками. Скрещивания показали, что свойство обусловлено геном. В настоящее время стараемся зафиксировать это свойство, но это затрудняется стерильностью и развитием малого количества рылец.

ВЗАИМОСВЯЗЬ МЕЖДУ ОБРАЗОВАНИЕМ ПЫЛЬЦЕВОЙ ТРУБКИ И МИКРОГАМЕТОГЕНЕЗОМ S. dulcamara L.

ДЬ. ПАЛ, Б. БАРНАБАШ

В процессе развития микрогаметофита S. dulcamara L. образование пыльцевой трубки в условиях **in vitro** может начаться в состоянии либо микроспоры, либо молодого гаметофита (двуклеточного пыльцевого зерна), а также зрелого гаметофита (трехклеточного пыльцевого зерна). Следовательно образование и рост пыльцевой трубки не зависит от состояния микрогаметогенеза. В процессе развития микрогаметофита одновременно совершаются два независимых друг от друга процесса; один из них — процесс развития, это микрогаметогенез, а другой — процесс роста, это процесс образования пыльцевой трубки.

УСКОРЕНИЕ ЯРОВИЗАЦИИ ПШЕНИЦЫ С ПОМОЩЬЮ СВОБОДНОГО ОТ 2-ХЛОРОЭТИЛФОСФОННОЙ КИСЛОТЫ ЭТИЛЕНА

А. КРОМИНСКИ, Б. РОЗЕЙ

Зерна озимой пшеницы, яровизированные в холодильнике при 2 °С выдерживались в растворах 50, 200 и 500 ppт. 2-хлороэтилфосфонной кислоты (СЕРА), освобождающей этилен. Ускорение яровизации наблюдалось у растений, обработанных холодом и раствором СЕРА.

РАЗМЕЩЕНИЕ УДОБРЕНИЯ И АБСОРБЦИЯ ФОСФАТА У ПОЛЕВЫХ КУЛЬТУР, ВЫРАЩЕННЫХ НА НЕИЗВЕСТКОВОЙ ПОЧВЕ МАНИТОБЫ

Й. П. КАЛРА

Эксперимент был проведен в оранжерее, с целью изучить влияние метода размещения удобрения на использование фосфата из неизвестковой почвы. Кристаллы P^{32}-меченого фосфата монокалия (диаметр 0,10—0,25 мм) размещались или помещались в форме гранул в маленькой полости в почве в середине сосуда приблизительно 1,4 см под семенами. 37,8; 27,2; 17,3 и 9,1 процентов фосфора было поглощено соответственно гречихой, рапсом, пшеницей и льном из одинаково размещенных кристаллов, и 24,2; 24,6; 14,2 и 5,0 процентов фосфора из гранул. Эти данные сравнивались с результатами, полученными ранее на известковой почве из Манитобы.

СОДЕРЖАНИЕ ФОСФОРА, ЛИПИДА И ФОСФОЛИПИДА В МИОФИБРИЛЛЯРНЫХ БЕЛКАХ

II. Содержание липида и фосфора в миозине

Ш. ФАЗЕКАШ, В. СЕКЕШИ-ХЕРМАН, Л. ВОДНЯНСКИ, Г. КАТОНА

В работе изучали гетерогенность миозина, а также содержание в нем фосфора и липида. Установлено, что содержание фосфора в миозине меняется в зависимости от метода препарации (3—4,5 г атом на 5. 10^5 г белка). Содержание фосфора во фракциях, подвергнутых хроматографии тоже меняется, и соответственно условиям выделения в зависимости от концентрации пирофосфата набирает данную концентрацию. Содержание липида в миозине было гораздо более высоким, чем 4%, что известно из литературных данных (**Lynn** 1965). Благодаря агрегации макромолекулы весь липид из миозина можно устранить только многократной обработкой **Chl—MeOH** в течение очень долгого времени, но подвергаясь диализу из молекулы медленно освобождается липид, большая часть которого появляется в диализирующей воде. Миозин, частично освобожденный из липида, дает ультрафиолетовый спектр, и спектр диффенреции-экстинкции можно зарисовать в отличии от сырого миозина, у которого этого сделать нельзя. На DEAE-целлюлозной колонке миозин можно разделять по крайней мере на 4 фракции, но только третья фракция показывает электрофоретически единственный компонент. Изучая отдельные фракции, подвергнутые хроматографии видно, что под влиянием диализа они еще дают липид, и даже крепкосвязанный остаток липида только в это время можно извлечь экстрагированием смесью **Chl : MeOH**. Свежеизолированные липиды миозина являются бесцветными жидкостями, которые на воздухе быстро подвергаются автоокислению и подвергнутые неспецифической полимеризации становятся смолоподобным материалом коричневого цвета. На пластинках ТЛЦ (гель силика) свежие липиды, освобожденные диализом дают 6—7 пятен, а хроматографированные фракции миозина только 2—3 пятна (на местах соотвествующим лецитину и кефалину), которые после автоокисления исчезают и показывают другую локализацию.

ИССЛЕДОВАНИЕ ПРОТОН-МАГНИТНОГО РЕЗОНАНСА НА РАСТИТЕЛЬНЫХ МАСЛАХ И СЕМЕНАХ

Л. ТОЛНАИ, К. ТОМПА

Измерения протон-магнитного резонанса проводились на семенах, а также экстрагированном масле трех масличных растений, с помощью ядро-магнетического резонансового спектрометра (ММР). Установлено, что имеющийся в наличии ММР-спектрометр является удобным для разделения ММР спектров протонов, находящихся либо в твердом материале (напр. в целлюлозе), либо в жидких или твердых жирах. Чувствительность оборудования достаточна для измерения одного семени, при достаточно большом отношении между знаком и шумом. Содержание свободной воды не мешает измерению масла.

РАЗЛИЧЕНИЕ ВИДОВ РОДА BRASSICA ФОТОМЕТРИЧЕСКИМ ИЗУЧЕНИЕМ КОМПЛЕКСА КРАСЯЩИХ ВЕЩЕСТВ СЕМЯН

Э. ПАПП

Комплекс красящих веществ семян рода **Brassica** различается по видам. Красящее вещество растворялось в 1,5 мольном растворе **NaOH**, показывая различные цвета. Цвет флуоресценции растворов и замоченных семян не отличался, но на дневном свете цвет растворов различался. Цвет растворов ещё лучше можно было различить на основании кривых, полученных с помощью спектрофотометра. На основе цвета растворов и кривых изученные виды и разновидности подразделяются на группы. Различение видов и разновидностей между группами оказалось возможным, а внутри группы — приблизительным, на основе упомянутых цветов и растворов спектрофотометрических кривых.

МОДЕЛЬ ПОГОДНЫХ УСЛОВИЙ ДЛЯ ИССЛЕДОВАНИЙ В ФИТОТРОНЕ

Я. ПЛЕТЧЕР

Автором излагаются главнейшие показатели строящегося в Мартонвашаре фитотрона, принадлежащего Сельскохозяйственному Научно-исследовательскому Институту Венгерской Академии Наук. Для моделирования элементов погодных условий использовалась серия данных Мартонвашарской Агрометеорологической Обсерватории, а также Всевенгерского Института Метеорологии. Подробно излагается метод соединения тригонометрической функции. Вычисления произведены на компьютере. Среди результатов приводится годовой ход 7 и 13 часового наблюдений, представляющих крайние показатели, а из суточных ходов приводятся январский и июньский, тоже представляющие крайние показатели в годовом отношении. Точность приближения соответствует установке элементов, программируемых в фитотроне.

ДИНАМИКА ЦВЕТЕНИЯ И ПЛОДОВИТОСТИ ПЕСТИКОВ У СОРТОВ ГРУШИ

Й. НЕКИ

Изучалась динамика цветения и плодовитости пестиков 12 сортов груши между 1968 и 1970 годами. Между сортами наблюдались различия по годам, по началу цветения, степени одновременного цветения и времени полного цветения. В среднем по изученным годам сорта требовали в сумме 3510,5 °C тепла, чтобы начать цвести. Пестики были плодовитыми в течение 2 или 3 дней, в зависимости от метеорологических факторов, «увядание» (побурение и высыхание) наступало скоро — через 1 или 2 дня. Раскрывание пыльников того же самого цветка в ясную погоду продолжалось в течение 1—2 дней, и в течение 4—5 дней в дождливую, холодную погоду. Сексуальная зрелость пестиков наступала на 1—4 дня раньше, чем у пыльников.

КОМПЕНСАЦИЯ ЭЛЕМЕНТОВ УРОЖАЯ У ЯРОВОГО ОВСА И ИХ СЕЛЕКЦИЯ

Я. СИРТЕШ

В опыте при рендомизированном распределении блоков, на высоком уровне удобрения почвы, методом линейного анализа регрессии изучались элементы урожая интенсивных сортов и линий ***Avena sativa*** L. а также их компенсация при ограниченной густоте стояния (в среднем 216 метелок/м^2). Используя ограниченную густоту стояния намеревались вынудить компенсационный механизм элементов урожая к действию. Линейная регрессия положительной корреляции была найдена между урожаем зерна и числом метелок на м^2, урожаем зерна и числом зерен на метелку, урожаем зерна и объемным весом (кг), а также между урожаем зерна и соломы. За исключением последнего варианта корреляция регрессии была на более низком уровне вариации, поэтому ее селекционная ценность оказалась ограниченной. Это значит, что самые высокие значения, показывающие корреляцию регрессии — 270 метелок/м^2, 85 зерен/металку и 45 кг объемный вес — должны быть приняты во внимание в опыте во время селекции, в противном случае урожай зерна может быть пониженным. Это заключение в сущности не является перспективным с точки зрения селекции. Положение благоприятнее в случае отношения между урожаем зерна и соломы потому, что их регрессия появилась на самом высоком уровне вариационной линии, обеспечивая значительную основу для выбора. Не было найдено регрессионной корреляции между урожаем зерна и весом зерна на метелку, а также между урожаем зерна и абсолютным весом. Благодаря ограниченной густоте стояния, вес зерна на метелку повысился в большой мере ($X = 2.65$ г.), и таким образом популяционный дефицит компенсировался.

ВЛИЯНИЕ РАЗЛИЧНОЙ ВЛАЖНОСТИ ПОВЕРХНОГО СЛОЯ ПОЧВЫ НА ПОГЛОЩЕНИЕ КУКУРУЗОЙ АЗОТА И ФОСФОРА

Й. ДОМБОВАРИ

Опыт в оранжерее был поставлен для того, чтобы определить влияние разного уровня влаги поверхностного слоя почвы на поглощение кукурузой N и P из удобрения; при этом нижняя часть корней находилась в чистой воде или в воде, содержающей N и P. На основе данных установлено, что уровень влажности почвы оказывал существенное влияние на содержание N, и незначительно влиял на содержание P в растениях. Влияние влажности почвы на процент N и P, поглощенных из удобрений было незначительным. Поглощение фосфора из удобрения, внесенного в почву, оказалось совсем небольшим в случае, когда растения были снабжены водой до нижней части корней. В растениях содержание азота, поглощенного из удобрения, оказалось сравнительно высоким в случае сухой почвы, хотя оно было в 2—5 раз меньше по сравнению с влажной почвой. Содержание влаги в почве не имело значительного влияния на поглощение N^{15} из раствора, но поглощение P увеличивалось с понижением уровня влажности в почве. При обработках, у которых NP дали и в почву и в воду, поглощение N^{15} зависило от влажности почвы в незначительной мере тогда, как поглощение P^{32} увеличилось в случае более низкого уровня влажности.

ИЗУЧЕНИЕ АБНОРМАЛЬНОГО РОСТА РАСТЕНИЙ

II. Структура галлов, вызванных насекомыми у Prosopis spicigera L.

Т. М. ВАРГЕЗЕ, С. ВАРМА

Изучалась анатомия стеблевых галлов, вызванных насекомыми у *Prosopis spicigera*. Заражение насекомыми происходит на ранних стадиях развития стебля. В стеблевых галлах насекомые обнаруживаются в корковой паренхиме, а также в медуллярных лучах. Зараженный стебель характеризуется ускоренной продукцией древесины. Образование васкулярных лучей прекратилось благодаря инфекции. Создание большего количества апотрахеальной паренхимы между трахеальными группами, а также образование лизигенных полостей оказались другими признаками, отмеченными в развитии стеблевых галлов.

ВЛИЯНИЕ СОРТА И АЗОТА НА ХАРАКТЕРИСТИКУ ТЕСТА, А ТАКЖЕ СОДЕРЖАНИЕ БЕЛКА У НЕСКОЛЬКИХ НОВЫХ ВЫСОКОУРОЖАЙНЫХ СОРТОВ ПШЕНИЦЫ

А. АУСТИН, Х. Д. СИНГ, Г. САДАСИВАН

Девять выведенных в настоящее время высокоурожайных сортов пшеницы, выращенных на трех уровнях азотного удобрения, изучались по содержанию белка и по характеристике теста, а именно время брожения теста, стабильность, площадь миксограммы, эластичность, угол брожения теста и высота пика. Результаты показывают, что сортовые различия были достоверными по всем признакам, тогда как влияние азота оказалось сигнификантным по высоте растений, эластичности и проценту белка. Рассматривая относительную характеристику теста различных сортов, Хеера, ХД. 1944 и ХД. 1949 очень надежными оказались время брожения, стабильность и площадь миксограммы, т. е. три признака, особенно важные с точки зрения хлебопекарного качества. Исследования корреляции показали, что содержание белка не влияет на качество теста.

РАСПРЕДЕЛЕНИЕ ОБЩЕГО СОДЕРЖАНИЯ B, Cu, Mn и Mo В ПРОФИЛЯХ НЕСКОЛЬКИХ ТИПОВ ПОЧВЫ НА МАЛОЙ ВЕНГЕРСКОЙ НИЗМЕННОСТИ,* И СВЯЗЬ ЕГО С НЕКОТОРЫМИ ПРИЗНАКАМИ ПОЧВЫ

Б. КЕРЕСТЕНЬ

Определены общее содержание бора, меди, марганца и молибдена в профилях гумусовых наносных, черноземно-луговых, типично луговых и навозно-луговых почв, образованных над обломочным конусом Дуная, а также в осушенной и разделенной на участки

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* Территория в северо-западной части Венгрии.

болотной местности. Во всех изученных типах почвы микроэлементы аккумулировались в верхнем слое, в 1,67—3,06 раз больше, чем в материнской или основной породе. Однако, благодаря стратификации аллювиального происхождения, аккумуляция микроэлементов была иногда найдена и в горизонтах В или С. Множественные регрессионные равенства показали, что содержания бора, меди и марганца было наивысшим в почвенных образцах, содержащих 8,5; 7,1 и 5,0 процентов органических веществ. В образцах, содержащих либо очень мало, либо очень много органических веществ, количество микроэлементов было одинаково малым. С другой стороны, содержание молибдена повысилось пропорционально повышению содержания органических веществ в почвенных образцах. Содержание меди было наивысшим в почвах, богатых устойчивым гумусом, а содержание молибдена — в богатых неустойчивым гумусом почвах.

В почвах, содержающих мало органических веществ, изученная биологическая и адсорбционная аккумуляция микроэлементов была одинаково сигнификантной, а в почвах, содержающих много органических веществ, содержание молибдена аккумулировалось главным образом биологически, а содержание меди и марганца — путем адсорбции. Содержание микроэлементов не влияло на условия растворимости, зависящие от химической реакции pH.

АНАТОМИЧЕСКИЕ ИССЛЕДОВАНИЯ ГАЛЛА НА КОРНЕ ЦИКОРИЯ (Cicherium intybus L.)

В. К. ШАРМА, А. К. СРИВАСТАВА

На корнях цикория образование галлов, вызванное инфекцией нематод корневого узла, началось пролиферацией паренхиматических тканей в центре, и явилось результатом гипертрофического роста. Вследствие увеличенного числа и размера паренхиматических клеток в коре, некоторые ткани погибли ввиду излишней мацерации и присоединились к перидерму. Перидерм окружил несколько живых паренхиматических клеток коры, вызвав таким образом образование больших полостей. Развитие трахеидов исходило из ряда поперечных начал, которые позже распространились везде, а на определенных местах спиральные трахеиды окружили живые паренхиматические клетки, создав таким образом большие полости. Спиральные, циркулярные, прямые, прямостоящие, послойные и изолированные, имеющие лопасти организации васкулярных элементов оказались главным признаком. Несколько этих трахеидов подверглись сегментации благодаря эксцессивной пролиферации окружающих паренхиматических клеток, поэтому фрагменты распространились везде в галле. В настоящее время ведется идентификация нематоды, связанной с образованием галлов в корнях цикории.

ВЛИЯНИЕ АГРОТЕХНИЧЕСКИХ ФАКТОРОВ НА ЭВАПОТРАНСПИРАЦИЮ РИСА

В. К. ВАМАДЕВАН

На протяжении двух вегетационных периодов риса сравнивались две популяции растений при двух глубинах воды с двумя дозами азота, и определялось их влияние на эвапотранспирацию риса. Эвапотранспирация измерялась установлением гальванизированных железных резервуаров на рисовом поле. В течение раннего периода роста риса увеличение водяного слоя вызывало сигнификантное увеличение эвапотранспирации. В позднем периоде разницы не наблюдалось. В обеих глубинах воды эвапотранспирация оказалась сигнификантно различной у двух разных популяций. Дозы азота в сущности не повлияли на эвапотранспирацию. Однако, наблюдалась тенденция, по которой высокое содержание азота увеличило эвапотранспирацию при глубине воды в 5 см. Противоположный эффект наблюдался при глубине воды в 20 см.

КОРРЕЛЯЦИЯ МЕЖДУ УРОЖАЕМ И ДРУГИМИ ПРИЗНАКАМИ В ЛИНИЯХ «А» У ПШЕНИЦЫ

Л. БАЛЛА

У многих гибридных комбинаций наблюдалась положительная корреляция между урожаем зерна и высотой растения, а также между урожаем зерна линии «А» и высотой его материнского растения. У таких комбинаций селекция на высоту может содействовать выделению высокоурожайных растений. Корреляция изменялась в зависимости от комбинации. Не было корреляции между урожаем зерна линий и вегетационным периодом, поэтому при наших условиях и у изученных комбинаций селекция на раннеспелость не влечет за собой снижения урожайности.

СОДЕРЖАНИЕ ХЛОРИСТЫХ ИОНОВ В НОРМАЛЬНОМ И ПАТОЛОГИЧЕСКОМ ОВЕЧЬЕМ МОЛОКЕ

А. ВАГНЕР

Автор занимается изучением содержания хлористых ионов в нормальном и патологическом овечьем молоке, которое подвергли тесту Schalm, меркуриметрическому титрованию и биометрическому исследованию. Определено среднее в 77 мг% для нормального и как предел 140 мг% или выше для патологического овечьего молока.

СОДЕРЖАНИЕ МАКРО - И МИКРОЭЛЕМЕНТОВ ЦВЕТКА

Й. ФРАНК, З. ЛЕНДВАИ

Авторы изучали содержание макро- и микроэлементов цветков отдельно в лепестках и плодолистиках. На основании количественного анализа они пытались объяснить важность различных элементов в формировании плодов. Обнаружено, что в цветках по сравнению с вегетативными органами, макроэлементы (исключая магний), а из микроэлементов железо и марганец уменьшались, тогда как содержание меди и цинка интенсивно увеличивалось. Колебания в поразительно низком соотношении Mn/Cu и Mn/Zn было вызвано в большинстве случаев скорее различиями в содержании меди и цинка, чем различиями в содержании марганца.

ДЕЙСТВИЕ ДОБАВЛЯЕМОГО К НЕПОЛНОЦЕННОМУ РАЦИОНУ DL-МЕТИОНИНА НА РОСТ РАСТУЩИХ ПТЕНЦОВ И ЭФФЕКТИВНОЕ УСВОЕНИЕ ИМИ КОРМА

АБД ЭЛЬ МЕГИД ДАРВИШ

Кормовой эксперимент с различными уровнями серной аминокислоты в рационе проведен на венгерских птенцах для того, чтобы выяснить подходящую в ней потребность В исследование были включены 160 птенцов (48 самцов и 112 самок), взятых с фермы сельскохозяйственного факультета Кестхея, Венгрия. Птицы были разделены на три группы. В то время, как первая группа получала основной рацион, второй и третьей группам в рацион добавляли DL-метионин и общее содержание серной аминокислоты было соответственно 0,9 и 1,2 процента. Эксперимент был начат, когда птенцы были 21-дневного возраста, и закончен, когда их возраст равнялся 81 дню; это время разделили на четыре периода продолжительностью в 15 дней каждый.

Получены следующие результаты: Потребность в количестве серной аминокислоты у птенцов в течение периода от 22- до 66-дневного возраста был более, чем 0,62, и менее, чем 1,2 процента, составив 0,9% в рационе для поддержания максимального переваривания пищи и максимального роста обоих полов. Избыток серной аминокислоты оказал подавляющее действие на рост и усвоение пищи. Потребность в серной аминокислоте у птенцов в раннем периоде роста была выше, чем в более позднем.

Добавление DL-метионина к неполноценному рациону в количестве 0,90% улучшало эффективность корма даже несмотря на то, что не было различий в ежедневном приросте, исключая третий период (51—66-дневный возраст), когда ежедневный прирост также увеличивался.

ОТНОШЕНИЕ МЕЖДУ ЭВАПОТРАНСПИРАЦИЕЙ И ОБРАЗОВАНИЕМ СУХОГО ВЕЩЕСТВА У РИСА

В. К. ВАМАДЕВАН

При большой густоте растений эффективность использования воды увеличилась при глубине воды в 5 и 20 см. Однако, содержание азота и глубина воды не повлияли на эффективность использования воды. Отношение между урожаем и эвапотранспирацией показало линейное увеличение эффективности использования воды при увеличении урожая.

ВЛИЯНИЕ КОЛХИЦИНА НА УРОВЕНЬ ПИГМЕНТОВ В ЛИСТЬЯХ РАСТЕНИЙ ЛЬНА

Р. ФАХМИ, Е. Н. САЛАМА

При обработке растений льна в период роста колхицином в количестве 50, 100 и 200 ppm в виде опрыскивания увеличился уровень хлорофилла-*а*, хлорофилла-*б* и ксантофилла. Содержание каротина осталось тем же самым, исключение составила концентрация 200 ppm. Положительное влияние колхицина на хлорофилл-*а* наблюдалось в течение всего вегетационного периода, а на хлорофилл-*б* и ксантофилл только до цветения. Обработка растений колхицином как в период роста, так и в период цветения положительно повлияла на уровень хлорофилла-*а* и *б*, каротина и ксантофилла. Этот эффект особенно выразился при 100 и 200 ppm колхицина, и мог наблюдаться до созревания. Колхицин, использованный три раза во время вегетационного периода, а именно: в период роста, цветения и плодоношения, не повлиял на вышеупомянутые пигменты, и обработка после цветения не вызвала дальнейшего увеличения уровня хлорофиллов.

ВЛИЯНИЕ ЦИНКА НА ОБРАЗОВАНИЕ КЛУБЕНЬКОВ И УРОЖАЙ СОИ (GLICYNE MAX.)

К. В. Б. Р. ТИЛАК, М. С. ГАНГВАР

Было обнаружено, что обычно при применении цинка урожай зерна уменьшался. Более высокие уровни цинка также угнетали образование клубеньков, синтез леггемоглобина и образование бактероидов.

Tervehdimme 80-vuotiasta professori Vilko A. Pesolaa!

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Greetings to the 175 years old Keszthely Georgikon

The Editors

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THE 175 YEARS OLD GEORGIKON

In the 18th century the ideas of the great French revolution spread in Hungary too. Especially those who had been in West-Europe and got acquainted with the ideas of the enlightenment desired the social and economic renewal of the country that lived in the backwater of feudalism. Reconciling the historical necessity with his own interests György Festetics, the enlightened magnate, recognized that the income of his estates could only be increased with the guidance of agricultural experts. It so happened that on the advice of the highly cultured scientist János Nagyváthy, and taking the instructions of Sámuel Tessedik, pioneer of agricultural education, in consideration in 1797 Festetics founded the first independent institution of higher agricultural education in Europe. The primary aim of the institution was to train farm managers for Festetics's estates, but soon a possibility was also given to other people to acquire an agricultural knowledge. On the suggestion of Károly Bulla, the first director, the school that had thus become a public institute was given the name "Georgicon", after Vergilius' famous didactic poem and the founder's first name.

Agricultural subjects were taught at the famous old European universities long before the 18th century. Agriculture was not, however, a separate field of the university education, it was only an encyclopedic subject completing the natural sciences. At that time the university professors thought that to deal with agricultural sciences was below their dignity, and the whole subject of agriculture only consisted, in essentials, of empirical, practical knowledge. Agriculture was torn away from the simple empirism of production by the establishment of independent agricultural high schools and also because agricultural branches and faculties were beginning to function at the universities. It was with this that the differentiation and development of agricultural sciences began, their special research methods were elaborated and the first scientific results revealing the correlations of cause and effect attained.

The activity of the Georgikon was thus not only a pioneer work in the field of agricultural higher education and agricultural research; it also promoted the development of agricultural sciences. As an agricultural high school it preceded Thaer's school established in 1802 at Celle, the Magyaróvár school (1818) and the Hohenheim (Stuttgart) Academy of Agriculture (1818).

Of the eight institutes of the Georgikon the Scientific Agricultural School was the first to begin functioning; it can be regarded as the predecessor of the present Faculty of Agronomy of the Keszthely University of Agricultural Sciences. The Scientific Agricultural School had three branches: agriculture, applied mathematics and physico-veterinary. Students were admitted after the final examination and studied over two or three years.

Fig. 1. The original building of the Georgikon

Susceptibility to everything new and the demand for a constant progress were pertinent to the spirit of the Georgikon. The teachers improved their scientific knowledge by various forms of self-education, by the modest research activity of those times, and — with the financial support of the founder — on foreign study tours, and endeavoured to keep education on an up-to-date level.

The history of the Georgikon, its development into an agricultural university was not unbroken during the past 175 years, nor was its functioning continuous. In 1848 the students announced that they wanted to take part in the Hungarian fight for freedom, therefore on May 25th of that year the Georgikon closed its door. After the failure of the revolution — as the Hapsburg tyranny could not overlook such a unanimous manifestation of patriotism —

the legal successor of the Georgikon was only allowed to begin functioning 27 years later, in 1865.

In accordance with the development of agricultural education in Hungary the Keszthely institution of agricultural education has undergone considerable changes. The present Keszthely Faculty of Agronomy of the University has developed from the Georgikon through the Agricultural High School, the Agricultural Academy, the College of Agriculture, the College of Agricultural Sciences and various other forms of education and organization.

The importance of an institution of higher education is not determined by its years of existence, but by the extent to which it has fulfilled its task of teaching and educating, and achieved the scientific development of its special field, and by the influence it exercised on its age. Looking at it in this way we can judge the Georgikon and its legal successor only by examining its role played in the education of Hungarian agricultural experts and in the development of agriculture.

The spirit and scientific level of the Georgikon were determined and shaped first of all by those who were in direct or indirect connection with the institute when it was established. The first to be mentioned of them was János Nagyváthy, the excellent agricultural expert who with his two volume work "Szorgalmatos mezei gazda" (The industrious farmer) published in 1791 caused great sensation in his days. After L. Mitterpacher's three volume work "*Elementa rei rusticae . . .*" published in the Latin language in 1779, it was the first Hungarian summarization containing the possibilities of rationalizing the Hungarian agriculture with regard to both plant growing and livestock breeding.

The influence which Sámuel Tessedik, founder of the Szarvas Agricultural School exercised on the Georgikon was of no less importance; he fought for the improvement of peasant farming by a many-sided agricultural activity including the amelioration of alkali soils, pastures and meadows.

At the time the Georgikon was founded, Ferenc Pethe, one of the most educated and open-minded agricultural experts and writers of Hungary was appointed assistant professor. During the four years of his professorate he taught the most up-to-date agricultural knowledge, which later was summarized in a three volume work "Pallérozott mezei gazdaság" (Refined agriculture). It was the work of a much experienced and well-read scientist who was thoroughly familiar with the soil conditions of Hungary and was thus able to adjust the results attained abroad to the Hungarian conditions. With his ten-course rotation — which was acknowledged by Thaer too — he laid the foundation of the rotation system in the model farm of the Georgikon. This was employed — with minor modifications — by the model farm up to the termination of the Georgikon in 1848. In the rotation row crops were followed by summer barley oversown with red clover, then by red clover, winter wheat, legumes and rye. In the seventh and eighth year the soil was left to rest and used for grazing. When the grassland was broken up flax or oats were sown, while in the last year rye was grown followed by buckwheat sown in its stubble.

The 51 years activity of the Georgikon was impressed by the pedagogic ideas of the first professors, first of all of Ferenc Pethe. And though not many data are available, we can establish that the Georgikon functioning at the end of the 18th century and in the first half of the 19th century was acknowledged by the public opinion both at home and abroad. The number of students registered in that period amounted to some 1500. Among the students, beside those who were given a scholarship by Festetics, there were many others both from Hungary and abroad.

The institution of agricultural education re-opening in 1865 followed the traditions of the Georgikon, and during its functioning of more than one hundred years exerted a continually increasing effect on the agricultural expert training and on the scientific development of agriculture.

The names and professional acknowledgements of the directors and department leaders often went beyond the borders of the country. The Keszthely institution of agricultural education has always occupied a distinguished place among the Hungarian institutions of agricultural higher education and research institutes.

It was within the frames of the institution that the Keszthely meteorological station began its regular observations in 1867. The experience gained there was directly utilized in the education.

In 1884 a seed testing unit and in 1885 an experiment station of agricultural chemistry were established. While the former had the aim of controlling the trade of seeds and protecting the interests of the farmers, foresters and gardeners, the task of the latter was to study the methods of cleaning the materials and products used in agriculture and provide expert advice promoting the development of agriculture and industry. The two stations were joined to the departments of plant growing and chemistry, respectively.

It would be difficult to enumerate everything that the Georgikon and its legal successor did in the field of agricultural research and development. The teachers and scientists of the agricultural higher education of those days often changed situation, so it would be difficult to link their activities and results closely to one or the other high-grade institution. For this very reason, without any claim for completeness, we should like to mention but a few of the scientists working at Keszthely in that period. Pál Sporzon, professor at the Department of Agriculture of the Technical University, was director of the Keszthely institution between 1867 and 1874; his literary activity as well as his work done in the development of agricultural professional training made his name widely known. Árpád Balás and Árpád Hensch did much for the improvement of field crop production. The book "Általános és különleges mezőgazdasági növénytermelés alapvonalai" (Elements of general and special agricultural crop production) (1876) written by Árpád Balás, and Hensch's work "Az okszerű talajművelés elmélete és gyakorlata" (Theory and practice of reasonable soil cultivation) (1885) had a considerable influence on the agriculture of those times.

Fig. 2. The central building of the Keszthely University of Agricultural Sciences

Mihály Soós was a pioneer of the practical protection of birds in Hungary. József Kukuljevics, also a teacher of the school, who wrote the first Hungarian book on bird protection, was his follower. It was at this school that Sándor Lovassy, pioneer of farm zoology performed his scientific activity. The activity of Imre Deininger, one of the undeservedly forgotten pioneers of Hungarian botanical research was of no less importance.

Among the famous teachers of the past we find Sándor Fáber, who first of all studied the questions of improving meadows and pastures and utilizing peaty areas. He was the first in Hungary to start the systematic breeding of soybean.

Gábor Groffits gained distinction in the field of agricultural building and construction by studying the modern equipment of stables and designing various silage systems.

But those living today who perform a teaching and educational activity, or as pensioners continue working in the field of agriculture must not be left unmentioned either. Those who between the two world wars and after the liberation of Hungary educated and are educating generations of agricultural engineers and who are working untiringly for the development of agriculture in Hungary.

The legal successor of the ancient Georgikon, the Keszthely Faculty of Agronomy of the University of Agricultural Sciences, Keszthely has been collaborating since 1970 with the Magyaróvár Faculty of Agronomy, successor of the ancient Academy of Magyaróvár in a new organizational form, within the framework of the same university. Furthermore, the earlier technical high-schools of Körmend and Nagykanizsa also belong to the Keszthely University as a college faculty for works engineers.

The whole activity of the new university established in Transdanubia is determined by the demands of the Hungarian society. In its teaching and educational work the demands required of the professionals of the socialist large farms, while in its research work a scientific research promoting the rapidly growing agricultural production, and expert advisory service closely connecting the theory and practice are the determinant factors. Therefore the Keszthely Faculty — like the whole university — has a task realized in three main activities: high-degree training and education of agricultural engineers, scientific research, and expert advice given to the farms.

In its teaching work the Keszthely Faculty educates general agricultural engineers, agricultural chemists, and works engineers for plant protection; while on postgraduate courses special engineers (of pig breeding, plant protection etc.). Besides, by giving doctor's degrees, and training aspirants it also prepares people for scientific life, apart from the practical requirements of production.

University level education develops only in a scientific environment, therefore scientific research will always be an integrate and indispensable part of the Faculty's activity. The Agricultural Research Institute of South-West Transdanubia merging in the Keszthely College nearly fifteen years ago has imposed considerable research tasks on the institution. The sections of the research institute amalgamated with the departments of the university bringing their research subjects and tasks with them.

The present research activity of the Faculty is fundamentally determined by a responsibility for and contribution to the programme researches. It is mainly through this that the scientific activity and international relations of the present Keszthely Faculty of Agronomy, successor of the Georgikon blossom out. The programme, or target plan ("Complex research of the questions of soil conservation", "Complex research of potato breeding and -growing" and "Management development in state- and cooperative farms") of the Faculty is realized with the contribution of almost all departments and of the other institutions of agricultural higher education and research in Hungary. Besides, the Faculty joins in other programmes too, as a collaborator. Apart from a considerable amount of so-called disciplinary research which improves the work of teaching, applied research is being carried on the commission of other organizations too.

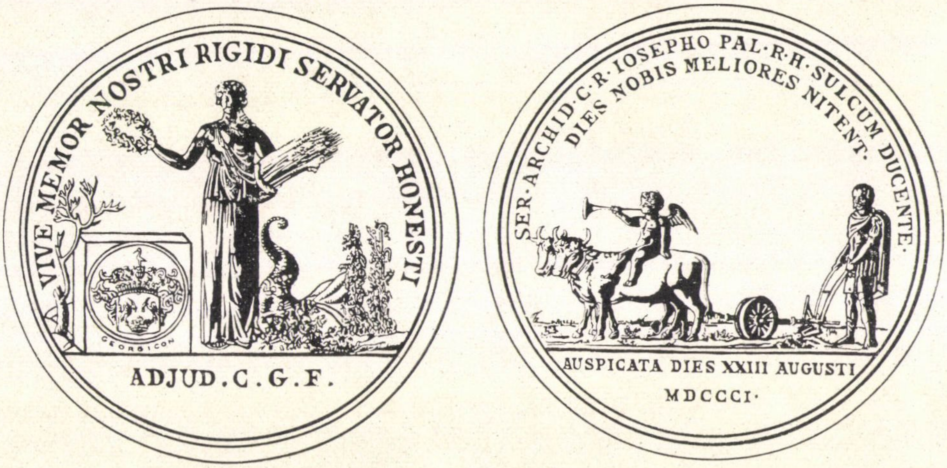

Fig. 3. The two sides of the Georgikon medal

The Georgikon was established in the age of the enlightenment and kept the flag of progress flying. Beside the preservation of the traditions of the past the successive generations have the task of building the future by promoting the development of agricultural sciences, not forgetting the legend on the Georgikon medal "Vive memor nostri rigidi servator honesti" (May the memory of those serving strictly and honestly live long).

A. Kováts

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THE SYNTHESIS OF TWO-ROWED MAIZE EARS

By

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We have developed two-rowed maize ears with unpaired spikelets by inbreeding and repeatedly crossing HEPPERLY's (1949) "odd-rowed" material with four-rowed lines. In the crosses the character proved to be genic controlled. Its fixation which is very difficult due to sterility and extraordinary few stigmas is in progress.

Introduction

In cultivated varieties of maize the number of kernel rows varies between 8 and 30 but in fasciated types where kernels do not form regular rows it can be much more. Several reports have also been published on homozygous four-rowed lines (DANIEL 1965) which can be of interest from the point of view of evolutional genetics (GALINAT 1971) but cannot be considered as normal in compliance with inheritance (DANIEL 1963, 1970). Generally spikelets are formed by a fertile and a sterile flower and stay in pairs on the cob; the row number is even. Accordingly in ears of the reported lines with four rows one pair of the spikelets faces the other: the two-ranked arrangement is common in the grasses and the question arises if it is possible to develop two-rowed maize ears with single spikelets.

Material and Method

HEPPERLY (1949) reported in his article "A corn with odd-rowed ears" on a maize material in which some ears were found to have spikelets not standing in pairs as normally but mostly singly, with the result that there occurred odd rows.

The row number of ears with single spikelets is generally only half of that of normal ears with single spikelets and, according to Hepperly, its inheritance is monogenic dominant. The material was thoroughly studied by WILCOX (1950) who discussed in detail its importance in evolution. Considering that even and odd rowed ears with single spikelets have the same probability to develop, he uses the more appropriate expression of "unpaired spikelets" instead of "odd-rowed". There did not occur any ears or tassels formed of only unpaired spikelets, on the other hand in half of the ears of the studied 13 families, according to families, 0—93 per cent of the individuals had kernel rows with unpaired spikelets. According to morphological and anatomical examinations the unpaired spikelet condition is not likely to be a teosine-like mutation but represents a more developed stage. Wilcox did not succeed in fixing the character in a homozygous state, either, but he suggests a more complicated inheritance.

In 1953 we received a sample of Hepperly's reported material — for which we are much obliged — and attempted to develop homozygous lines with unpaired spikelets by inbreeding. A selection combined with self-fertilization through 7 generations proved unsuccessful, in 1961 we began to cross our four-rowed lines (Fig. 1) with the heterozygous material and selfed or backcrossed the hybrids. In the selected progenies the frequency of individuals with unpaired spikelets greatly increased and at the same time the row number decreased. Unfortunately sterile individuals or ones with only a few stigmas are rather frequent making propagation of the material with unpaired spikelets very difficult. Pollen development is, however, quite normal.

Results

Two two-rowed maize ears with nearly unpaired spikelets were found in 1966 among individuals with four-rowed ears and unpaired spikelets (Figs 2, 3).

Data of some "lines" with characteristically low row number in 1971 (ears with more than 30 per cent of paired spikelets are underlined):

A) 2, 4, 4, 4, 4, 2, $\underline{8}$, 4-2, 4
B) 2, $\underline{8}$-$\underline{4}$, $\underline{8}$-$\underline{4}$, 5-2, 4-2, 4-2, 4-2
C) (4)-2, 2, 4
D) 4-2, 4-2, $\underline{8}$-$\underline{4}$, $\underline{8}$-$\underline{4}$, 4-2
E) 4-2, 4-2, 4, 4-2, 4, 4, 4-2, 4, 3-2, $\underline{8}$-$\underline{4}$, 4-2, $\underline{8}$

In agreement with Hepperly's (1949) statement the row number of ears with paired spikelets was found to be twice as much as that in ears with unpaired spikelets. At least in one of a generally four-rowed family (n = 88) the character of unpaired spikelets is probably fixed. The genetical analysis is in progress.

At last in 1970 we succeeded in crossing a four-rowed homozygous individual with paired spikelets (P4) with a probably heterozygous individual with two-rowed ears and unpaired spikelets (E), $/[(P4 \times E_{S8})\ S5 \times P4]\ S1/$. The ear of the two-rowed pollen parent remained stunted and did not form kernels. In 1971 we studied the ears of 70 S_0 plants (Table 1, Fig. 4). In rough sorting 25 per cent of the ears have four, 25 per cent intermediate and 50 per cent two kernel rows; 25 per cent have paired and 75 per cent unpaired spikelets. It is worth noting that the four-rowed ears have unpaired spikelets while in one-sixth of the two-rowed ears there is a remarkably high percentage of paired spikelets. Sorting into row number was performed on the basis of general impression. One member of the paired spikelets in the two-rowed material is generally much smaller than the other; no attention was paid to it, however, when sorting into kernel row number. Unfortunately the seed set in the selfed ears was extremely poor, even in the P4 line which generally has good setting ability.

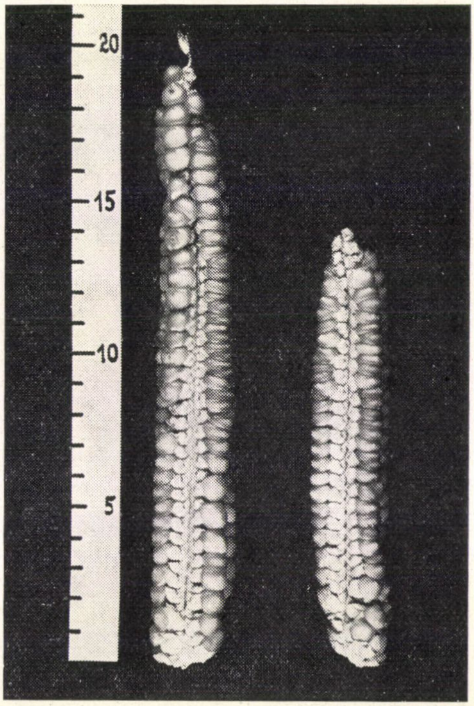

Fig. 1. Four-rowed maize ears with paired spikelets

Table 1

Distribution of the first generation /{$P4 \times [(P4 \times E_{S8})\ S5 \times P4]\ S1\}\ S_0$/ *of an inbred plant with four-rowed ear and paired spikelets (P4) and a heterozygous plant with two-rowed ear and unpaired spikelets (E) according to number of kernel rows and position of spikelets*

Number of kernel rows	Arrangement of spikelets in the ear							Total
	Paired	Mixed; percentage of unpaired spikelets					Un-paired	
		<20	20–39	40–59	60–79	≧80		
4						2	14	16
4—2	3		1	1		3		8
(4)—2		4		2		1	3	10
2		1	1	4	5	24	1	36
Total	3	5	2	7	5	30	18	70

Numerous non-inheritable abnormal forms are known in maize ears and if we consider that the ear of the four-rowed parent in the cross, though of constant form, is yet an anomaly, the often undeveloped two-rowed ear

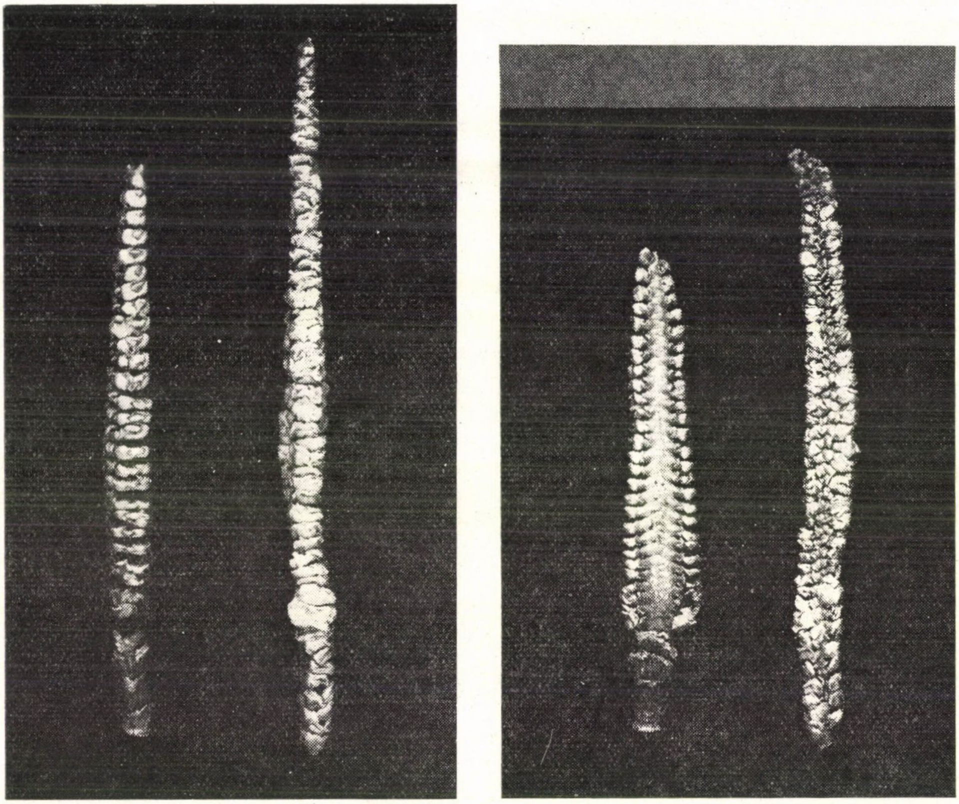

Fig. 2. Two two-rowed maize ears with almost entirely unpaired spikelets; side and top view

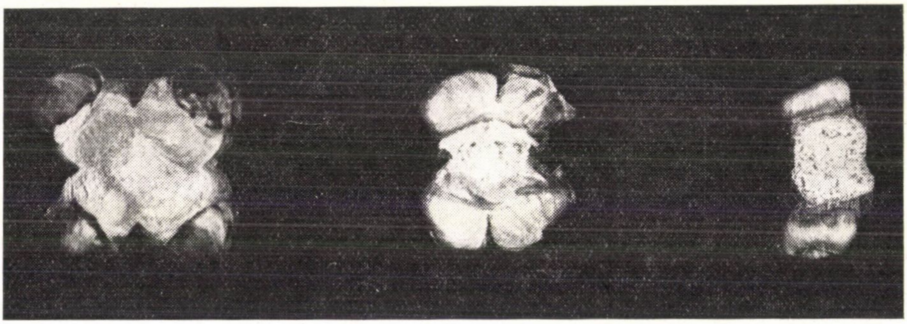

Fig. 3. Cross section of four-rowed maize ears with unpaired, that of four-rowed ears with paired and that of two-rowed ears with unpaired spikelets

with unpaired spikelets and often without any stigma, can easily be taken for an abnormal, genotypically not determined formation. The cross we outlined here clearly shows that the two-rowed ear types with unpaired spike-

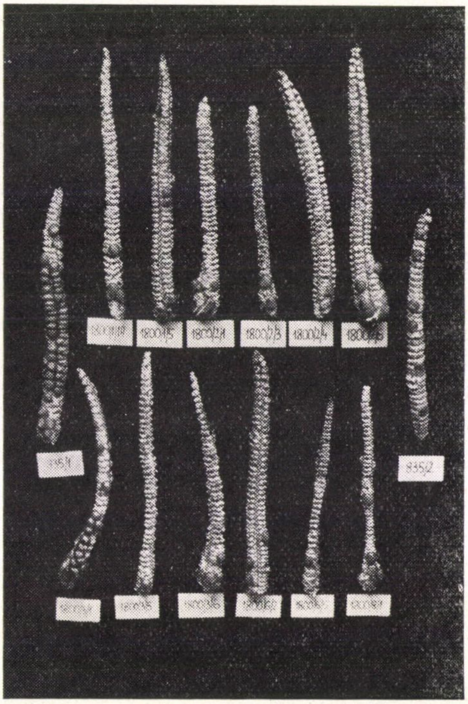

Fig. 4. Self-fertilized ears of the first generation of a cross between normal four-rowed line (835/1, 835/2) and a two-rowed individual with unpaired spikelets (Table 1)

lets found in our tests are genic determined. The observed segregation ratio is difficult to explain. Besides difficulties in the survey it is very probable that penetrance and expressivity problems also contribute to make the determination of inheritance difficult. However, it is quite clear that neither the inheritance of row number nor that of unpaired spikelets is monogenic dominant.

Discussion

Though some authors are still dissatisfied with the evidence presented (Galinat 1971) palynological (Bartlett—Barghoorn—Berger, 1969) and archeological findings acceptably prove that modern cultivated maize derives from an ancient maize with ears of some cm length, thin rachis, paired spikelets on relatively long pedicels, and glumes covering the kernels. It is very likely that already in historical times it crossed with some of its relatives still found in nature. Crossing had a shortening effect on the pedicel, the glume also became shorter and its butt thicker leaving a great part of the kernels naked (MANGELSDORF 1961). Compared to the tripsacoid unpaired spikelets maize has maintained the paired spikelet condition, though unpaired spikelet mutants sporadically occur. GALINAT (1971) deals with their importance in evolution. In maize the spikelets in the ear and those in the tassel stand in pairs while spikelets in teosine stand in pairs in the tassel but single in the female inflorescence. The pd allele (probably on chromosomes 3 and 7) determining the unpaired spikelet condition in teosine is generally recessive in contrast to the Pd allele determining the paired spikelet condition in maize. Mutants to unpaired spikelets in maize are not stable in most genetical backgrounds, their dominance is relative. GALINAT wants to fix HEPPERLY's unpaired spikelet mutation by using a certain teosine chromosome. We hope to have succeeded in that by using four-rowed lines. Genetical test are going on.

The two-rowed maize ear is without doubt a tripsacoid character but in contrast to it its unpaired spikelet condition is dominant or partially dominant and by no means recessive; the cob has retained the characteristics of maize and in its development no further teosine introgression could have a part. We hope to develop homozygous lines by means of better cultural methods, selection and perhaps intercrossing.

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RELATIONSHIP BETWEEN MICRO-GAMETOGENESIS AND POLLEN TUBE FORMATION IN SOLANUM DULCAMARA L.

By

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During the development of the micro-gametophyte of *Solanum dulcamara* L. the in vitro formation of the pollen tube may equally begin at the stage of microspore, young gametophyte (two-cell pollen grain) or mature gametophyte (three-cell pollen grain). Thus the formation and growth of the pollen tube is independent of the different stages of micro-gametogenesis. During the organization of the micro-gametophyte two separate processes take place at the same time; one of them is developmental, this is the micro-gametogenesis, the other is a process of growth, this is the tube formation.

Introduction

Investigations into the fertilization process of plants were primarily aimed at determining the growth rate of pollen tube formation. The question was, namely, how long after the pollination the different stages of the fertilization process set in. The time between pollen grains reaching the stigma and the pollen tube beginning to develop was determined in various plant species (ARTSCHWAGER—BRANDES—STARRETT 1929, HALLOCK 1930, ARTSCHWAGER—STARRETT 1933, PODDUBNAYA-ARNOLDI—DIANOWA 1934, RANDOLPH 1936, EIGSTI 1937, POPE 1937, ARTSCHWAGER—MCGUIRE 1949 in: MAHESHWARI 1950; ELITROPI 1958, KAVETZKA 1958, MACKEY 1959, MARTIN 1959, KONSTANTINOV 1960). According to the authors' findings this process may begin immediately, or may even be delayed for two or three days. Contradictory results can be found concerning the controllability, acceleration and slowing down of the process as well (BATIKYAN—CHOLOHYAN 1958, ELITROPI 1958, SIZOVA 1958, SHCHEDRINA 1959, DZYUBENKO 1960, HOSHIKAWA 1960, KONSTANTINOV 1960, OKSIYUK—HUDYAK 1961, SMITH 1963).

These investigations did not, however, include the question of what stage of development the pollen grain was at when reaching the stigma. Namely, it is not known whether there is any relationship between the different stages of micro-gametogenesis and the beginning of pollen tube formation. It is not known whether the beginning of pollen tube formation postulates a definite stage of development in the process of micro-gametogenesis, or may set in at any stage. It is known that pollen tube formation may begin after the first and second division taking place in the course of the gametogenesis, but no data are available on its possible occurrence at the microspore stage. In vitro studies have therefore been made to find out whether the pollen tube formation begins at the stage of microspore, young gametophyte (two-cell pollen grain) or mature gametophyte (three-cell pollen grain), or whether it may occur at any stage of development.

Material and Method

Pollen grains of different development stage in differently ripe anthers of *S. dulcamara* L. were used in the investigations. The tube formation of pollen grains was induced on a culture medium prepared from the mixture of 10 ml 35 per cent saccharose solution and 0.1 ml 1 per cent borid acid. The optimum concentration of saccharose was determined from pollen tube growth found with various concentrations of saccharose solution.

Two drops of each culture medium were spread over slides and sprinkled with a few pollen grains taken from anthers of different ripeness. We placed the slides in Petri-dishes on cotton wool saturated by water thus ensuring the adequate moisture content during the pollen tube growth. Then the Petri-dishes were covered and placed in a thermostat at a temperature of 30°C. Pollen tube primordia appeared after two hours; 100 per cent tube growth was only obtained, however, with five hours of incubation. Pollen grains which developed tubes were stained with carmine acetic acid. As a result of staining the nuclei of the vegetative and generative cells as well as the micro-gametes turned red, while the cytoplasms of the cells only coloured slightly. The investigations were made with a Leitz Ortholux microscope on several thousands of preparations.

Results

In anthers of different ripeness the development stage of the pollen grains is also different. Some of them are in a microspore state, while others at the two-cell or even three-cell stage of development. The most frequent state depends on the development stage of the anther, but pollen grains at the two other development stages also occur though to a less extent. Most of the pollen grains in ripe anthers are at the two-cell and three-cell stage, respectively. If the content of a less ripe anther is spread over a culture medium, then all stages of development occur. After the pollen tubes were grown the different stages of development could easily be recognized by staining with carmine acetic acid, as the nuclei of the vegetative and generative cells as well as the microgametes turned bright red.

Tube growth of microspore. If the culture medium smeared over the slide is sprinkled with the content of a young, less ripe anther, then a relatively large amount of microspores can be found in it. During the time of incubation these microspores grow tubes without the process of development in them having advanced. Thus, the microspores of Angiospermae — like those of the dioecious bryium and heterospore ferns — are able to grow tubes. Microspores growing tubes may be of two kinds depending on the position of the nucleus.

Fig. 1. Tube growth of microspore (*a* the nucleus is in the microspore, *b* the nucleus is in the tube of the microspore)

The single nucleus of the microspore may be located in the microspore (Fig. 1), or may get — due probably to the cyclosis — into the tube (Fig. 2). It is not known whether the internal development of the microspore stops or not during the tube growth, since staining with carmine acetic acid takes place after the tube growth. There may even occur microspores which grow tubes during the time of incubation and develop into two-cell pollen grains. Once stained with carmine acetic acid they are regarded as two-cell pollen grains having grown tubes. In the case of microspores growing tubes, the development of the generative and vegetative cells, that is the first division of the microspore, may occur either in the microspore proper or in the tube of the microspore, depending on whether at the time of the tube growth the nucleus of the microspore is in the microspore or has got into the tube.

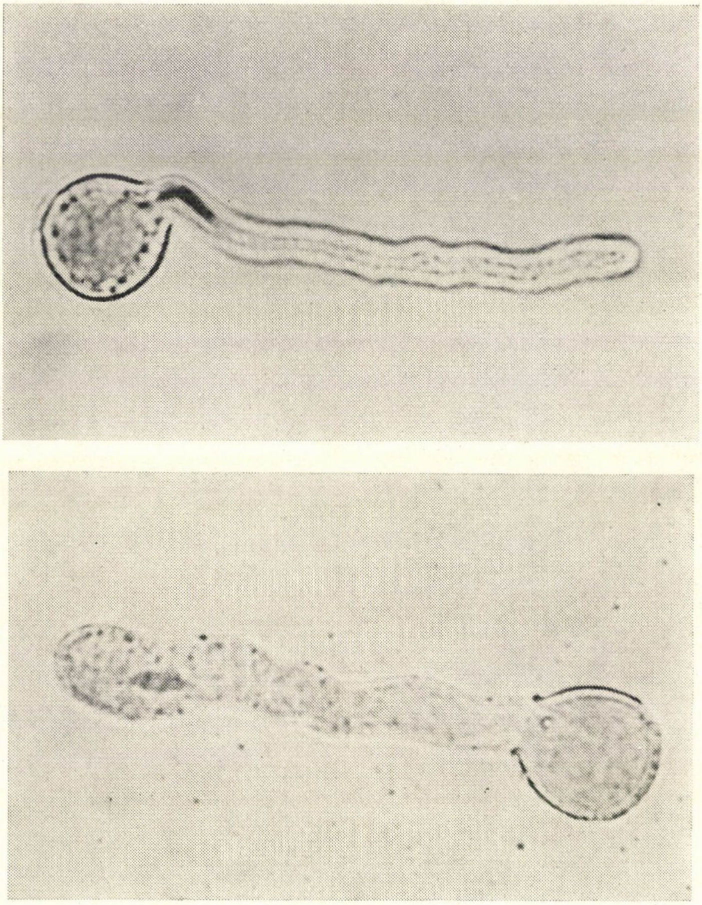

Fig. 2. Tube growth of microspore (*a* the nucleus enters the tube of the microspore, *b* the nucleus is in the tube of the microspore)

Tube growth of the young gametophyte (two-cell pollen grain). In ripe anthers most of the pollen grains have two cells. The young gametophyte (two-cell pollen grain) is also able to grow a tube. There are three types depending — in this case too — on the location of the nuclei of the vegetative and generative cells: both nuclei are in the pollen grain (Fig. 3), both nuclei are in the pollen tube, and one of the nuclei is in the pollen grain while the other in the pollen tube. The latter case may have two types again according to which one of the nuclei is in the pollen grain and which of them in the pollen tube. All three types occur with about the same frequency. It may happen, here too, that during the time of incubation the two-cell pollen grain develops into a three-cell one while growing a tube, and after having been stained with carmine acetic acid is considered a three-cell pollen grain having grown

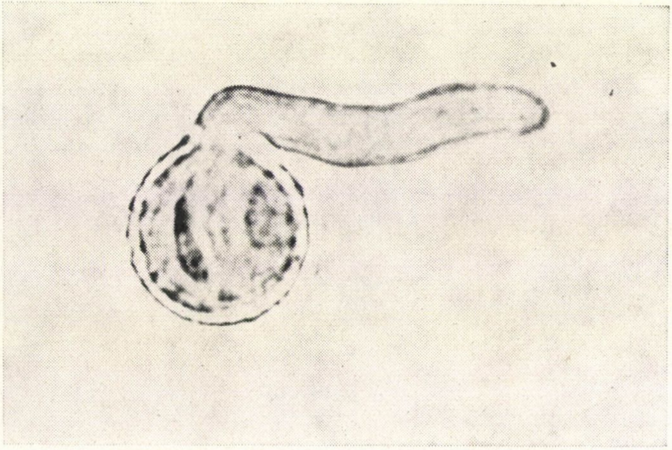

Fig. 3. Tube growth of the young gametophyte (two-cell pollen grain) (the nucleus of the vegetative cell and the generative cell are in the pollen grain)

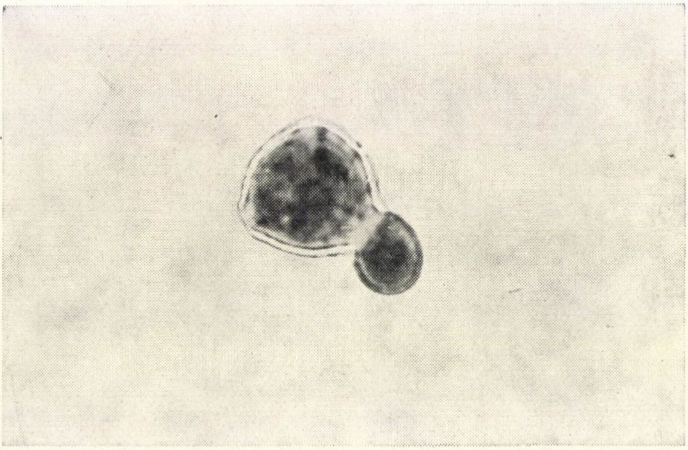

Fig. 4. Tube growth of fully developed gametophyte (three-cell pollen grain) (the nucleus of the vegetative cell is in the pollen tube, the two microgametes are in the pollen grain)

a tube. In the case of two-cell pollen grains growing tubes, the second division of the microspore, that is the formation of microgametes, may equally occur in the pollen grain and in the pollen tube depending on where the generative cell is at the time of division.

Tube growth of fully developed gametophytes (three-cell pollen grains). In ripe anthers a part of the pollen grains have three cells. In such pollen grains the division of the generative cell too has already been completed, and two microgametes, each of the value of a cell possessing a relatively small amount of cytoplasm, have been produced. Fully developed gametophytes (three-cell pollen grains) are also able to grow tubes (Fig. 4). In this case too, as in the case of two-cell pollen grains, three types occur according to the location of the nucleus in the vegetative cell and of the two micro-gametes. In the case of three-cell pollen grains growing tubes the first and second division during the micro-gametogenesis always take place in the microspore.

Discussion

During the development of the micro-gametophytes of *S. dulcamara* L. tube formation in vitro may begin either in the microspore, or in the young (two-cell pollen grain) or mature (three-cell pollen grain) gametophyte. Tube formation and growth are thus independent of the different phases of micro-gametogenesis. Tube formation may begin at any of the three stages of development. In our opinion micro-gametogenesis — the inner development of the microspore — does not stop during the tube growth, since in such cases when the generative cell gets into the pollen tube the microgametes too are formed there. The problem cannot, however, be solved on the basis of our investigations, as staining with carmine acetic acid was carried out after tube formation had been completed. By the staining we fixed a given stage of development. We do not think it probable either that during the development of microgametes the growth of the pollen tube would stop or even slow down. This is supported by our finding that tube formation and growth are independent of the different phases of micro-gametogenesis. In our opinion two simultaneous processes take place during the organization of the micro-gametophyte. One of them is a developmental (this is the micro-gametogenesis), the other a growth process (this is the tube formation and growth).

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ACCELERATION OF VERNALIZATION IN WHEAT BY 2-CHLOROETHYLPHOSPHONIC ACID-RELEASED ETHYLENE

By

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Kernels of winter wheat vernalized in the refrigerator at 2°C were exposed to the continuous presence of 50, 200 and 500 ppm solutions of 2-chloroethylphosphonic acid (CEPA), an ethylene releasing compound. The enhancement of vernalization was observed in plants subjected to both cold and CEPA treatment.

Introduction

Studies of the physiological roles of ethylene have revealed the effectiveness of this unique gaseous plant growth hormone in affecting the growth and development of many species also within the *Gramineae* family. ROBERTS (1951) has reported the growth inhibition of both shoots and root of wheat (*Triticum dicoccum* Schubler) seedlings exposed to gaseous ethylene.

2-chloroethylphosphonic acid (ethephone; abbr. CEPA), which exerts its physiological effects upon ethylene yielding decomposition within the plant tissues (COOKE—RANDALL 1968, WARNER—LEOPOLD 1969, YAMAGUCHI—WANG CHU—YANG 1971, YANG 1969), has also been shown to reduce the culm growth of various cereals (Amchem Products 1969) as well as of several species of ***Poa***, ***Festuca***, ***Agrostis***, ***Lolium*** and ***Phleum*** (ANDEL 1970). In the latter report, it has been observed that in non-vernalized, CEPA-treated seedlings of *Poa*, *Festuca* and *Agrostis* the temporary reduction of vegetative growth was followed by the induction of stem formation, however, no data have been presented on the earing or flowering of the treated plants.

Every plant produces ethylene and is influenced by ethylene at some stage of its ontogeny (PRATT—GOESCHL 1969); yet neither ethylene nor ethylene releasing CEPA appear to have been previously tested for their vernalization activity. We were thus prompted to examine whether CEPA may affect vernalization in wheat.

Material and Method

Kernels of winter wheat (*Triticum aestivum* ssp. *vulgare*) cv. Leszczynska Wczesna were germinated at 22°C in distilled water (control I) or in CEPA solutions of 50, 200 and 500 ppm. After 24 hrs the kernels were transferred to the refrigerator where they were kept for

40 days at 2 ± 1°C. Every fifth day the water content of the kernels was adjusted to 45%. The kernels were sown on May 24, 1971, into plant beds of 1.75 sq. m each, situated in the experimental field of the Copernicus University Agricultural Experimental Station, Piwnice nr. Torun, in northwestern-central Poland, in four replications. Kernels previously soaked for 24 hrs at a temperature of 22°C in distilled water (control II) or in CEPA solutions of 50 and 500 ppm (control III) were sown simultaneously in the same experimental field. Beginning with the appearance of the first ear among the plants of control I, the number of eared plants were recorded, the observations being made every week until no changes in heading were noted.

Results

No ear formation occurred in any of the plots sown with unvernalized wheat, i.e. in controls II and III, regardless of CEPA treatment of control III kernels. The noticeable increase in the number of headed plants, however, took place in the plants subjected to both vernalization and CEPA treatment as compared to the vernalized but untreated plants of control I (Table 1 and Fig. 1). These results show that CEPA-released ethylene cannot substitute the effect of cold treatment; ethylene action, therefore, should be regarded as made possible by the vernalization treatment.

The considerable, reaching as much as 8 to 53-fold, increase in the number of eared plants observed on the 70th day after sowing indicates that CEPA-treated plants attained the generative stage earlier. Thus the activity of the CEPA-released ethylene may be accounted for by the modification of

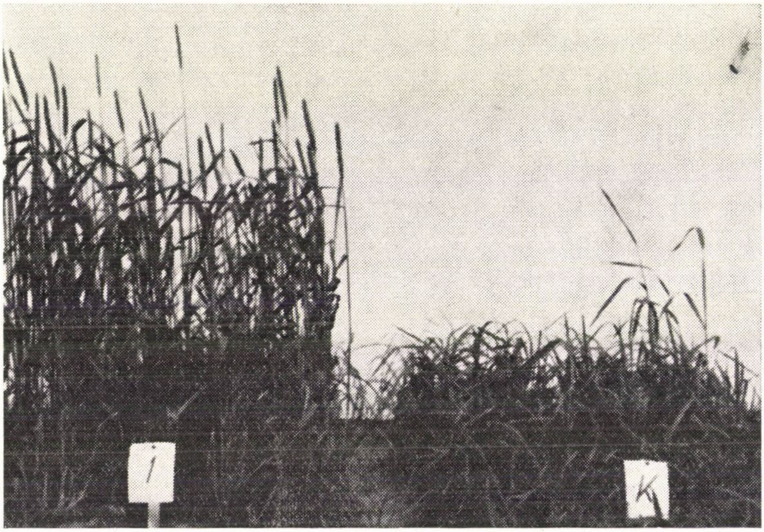

Fig. 1. Acceleration of vernalization in winter wheat cv. Leszczynska Wczesna caused by 2-chloroethylphosphonic acid (CEPA), an ethylene releasing compound. Photo taken on the 63rd day after sowing. (K = vernalized plants referred in text as control I, 1 = vernalized plants subjected to the CEPA-treatment [50 ppm])

the metabolic processes which follow the vernalization stimulus and are involved in the transformation of a vegetative to a reproductive meristems as well as the acceleration of such a transformation. Evidence, other than presented here, for either mechanism is lacking at the present time.

Table 1

Effect of joint vernalization and CEPA treatments on the number of headed plants of Leszczynska Wczesna winter wheat. In percent of the vernalized but untreated plants referred to in the text as control I

Days after sowing	CEPA, ppm		
	50	200	500
70*	5352.8	1961.1	861.1
77	1023.0	201.9	170.9
84	702.1	391.2	159.3
92	706.1	404.5	185.0
98	543.7	423.2	179.1

* August 2, 1971.

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FERTILIZER PLACEMENT AND PHOSPHATE ABSORPTION BY FIELD CROPS GROWN ON A NON-CALCAREOUS MANITOBA SOIL

By

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A glasshouse experiment was conducted to study the influence of method of fertilizer placement on phosphate utilization from a non-calcareous soil. P^{32}-tagged monopotassium phosphate crystals (0.10—0.25 mm in diameter) were spread or placed as a pellet in a small cavity in the soil in the centre of the pot about 1.4 cm below the seeds. Buckwheat, rape, wheat and flax absorbed 37.8, 27.2, 17.3 and 9.1% phosphorus from the uniformly-spread crystals and 24.2, 24.6, 14.2 and 5.0% phosphorus, respectively from the pellet. These data have been compared with the earlier results with calcareous soils from Manitoba.

Introduction

In earlier studies it was found that field crops differ in their phosphate uptake efficiencies (Kalra 1965, 1971a; Kalra—Soper 1968) and also that these efficiencies are influenced by the mode of application and phosphate carrier (Kalra 1971b, Soper—Kalra 1969). These studies were conducted on moderately calcareous (Kalra—Soper 1968, Soper—Kalra 1969), strongly calcareous (Kalra 1971a, 1971b) and very strongly calcareous (Kalra 1971b) soils.

Efficiency of the method of phosphatic fertilizer application has been reported to be affected by the calcareous nature of the soil. It has been a common observation that the chemical characteristics of phosphate fertilizers assume greater importance on calcareous soils than on acid soils (Web *et al.* 1961b). For example, Web *et al.* (1961a) found that for oats the degree of phosphorus water solubility assumed greater significance on calcareous soils while placement effects were of greater significance on acid soils. In greenhouse experiments Speer *et al.* (1951) compared 11 phosphatic fertilizers for their availability to Black Valentine string beans on an acidic sandy loam (pH = 6.0, citrate-extractable P = 14 ppm) and an alkaline, calcareous black clay (pH = 8.1, citrate-extractable P = 40 ppm, $CaCO_3 = 8\%$). Two methods of fertilizer application were compared. For banding, the fertilizer was applied in a small localized area in the centre of the pot about two inches below seed level. For the other method, the fertilizer was intimately mixed with the soil. Efficiency of these methods varied for the two soils. It was reported that on calcareous soil relatively water soluble fertilizers showed significant merit. Apparently, uptake of applied phosphorus depends to a large extent upon the pH and the $CaCO_3$ content of the soil. Therefore, this research was designed to investigate whether the differences found on calcareous soils are also observed on a non-calcareous soil.

Material and Method

The experiment was conducted on a Wellwood clay loam. These well-drained Black soils are developed on thin medium textured lacustrine deposits underlain by stratified sand at ca. 0.8 to 1.3 m below the surface (EHRLICH *et al.* 1957). The soils, representative of the 0—15 cm depth, were collected and analyzed as given earlier (KALRA—SOPER 1968). Some of the characteristics are given in Table 1.

Table 1

Soil characteristics

pH	6.4
Conductivity	0.3 mmhos/cm
Organic matter	6.1%
$CaCO_3$ equivalent	0.0%
Nitrate-N	8.3 ppm
Easily soluble P	18.1 ppm
Exchangeable K	310 ppm
Moisture content at field capacity	28.1%

Two kilograms of the air-dry soil that passed through a 6-mm screen were placed in 2.3-litre porcelain glazed pots (diameter = 14 cm). Wheat (*Triticum aestivum* L. "Manitou"), flax (*Linum usitatissimum* L. "Redwood"), rape (*Brassica napus* L. "Tanka", Argentine type) and buckwheat (*Fagopyrum esculentum* Moench) were the test crops. The carrier-free P^{32} (in the form of H_3PO_4) was obtained from the Atomic Energy of Canada Ltd., Ottawa. Radioactive KH_2PO_4 crystals (diameter 0.10 to 0.25 mm) were prepared and applied as "spread" and "pellet" (SOPER—KALRA 1969), supplying 20 mg P^{31} and 10 μc P^{32} to each pot. There were 3 replications. A uniform stand was obtained by thinning. Other details are given in the earlier two publications (KALRA—SOPER 1968, SOPER—KALRA 1969).

The experiment was conducted in a permanent greenhouse. Light intensity at ca. 40 cm above the plants was 16,000 lux to give a daily 16-hour photoperiod. The temperature was about 21°C (70°F) during the day and 16°C (61°F) at night. The plants were harvested 52 days after seeding, digested with an $HNO_3—H_2SO_4—HClO_4$ mixture (10 : 1 : 4 v/v/v) and analyzed as given in an earlier paper (KALRA—SOPER 1968).

Results

The "t" test showed that the amounts of phosphorus absorbed from the soil as well as the plant yields, from the two methods of fertilizer application, were not significant (Table 2).

Table 2

Influence of mode of application of fertilizer on yield and phosphorus utilization of the above-ground portions of four field crops

Crop	Yield g/pot		Utilization of fertilizer p %		Uptake of soil p mg/pot		"A" value ppm	
	Spread	Pellet	Spread	Pellet	Spread	Spread	Pellet	Pellet
Buckwheat	5.97	6.17	37.75	24.17	8.79	9.74	11.64	22.08
Rape	4.10	3.96	27.24	24.63	8.36	8.21	15.41	16.69
Wheat	3.06	3.10	17.33	14.19	4.05	5.64	11.70	19.93*
Flax	1.87	2.01	9.09	4.98*	2.92	3.61	16.32	37.12*

* Significant at 5% level.

In view of the earlier data for a moderately calcareous soil (Soper—Kalra 1969) and the present results for a non-calcareous soil, it is evident that the efficiency of the two methods of application of phosphatic-fertilizer crystals depends, amongst other factors, upon the pH and the content of calcareous material in the soil. For buckwheat and rape in calcareous soil, phosphorus from the "pellet" treatment was more available than from the "spread" treatment but the opposite was found in the non-calcareous soil. In the case of flax and a cereal, phosphorus from the "pellet" was more available in the non-calcareous soil than the calcareous soil. Although there was a higher uptake of fertilizer from the "spread" treatment than from the "pellet" method in all crops, the difference was significant only in flax.

In an earlier experiment (Soper—Kalra 1969) it was found that fertilizer phosphorus absorption decreased to one-sixth for flax and one-third for a cereal (oats) when the method was changed for applying crystals from "spread" to "pellet." A decrease in fertilizer uptake by flax and a cereal (wheat) was found in the non-calcareous soil also but it was not as large as in the calcareous soil.

On calcareous soils, the uptake of phosphate fertilizer by flax increased with an increase in the area of fertilizer placement (Soper—Kalra 1969). The differences between the efficiencies of the two methods of fertilizer application in supplying phosphorus to flax and the cereal were greater on the calcareous soil than on the non-calcareous soil. Rape and buckwheat absorbed more phosphorus from the crystals placed in the centre of the pot than from the crystals spread uniformly as a narrow band in the calcareous soil but the opposite was found in the non-calcareous soil.

Several factors may be responsible for the observed variations among calcareous and non-calcareous soils. These variations may be at least partly due to the movement of phosphorus from the place of application. Lewis—

Racz (1969) reported that the extent of phosphorus movement from the application site of a pellet was greater in non-calcareous soils than in calcareous soils for mono-ammonium phosphate. Moreover, the rate of phosphorus movement was also rapid in the former soil. The pH and the cations present in the soil would determine the type of reaction that would occur upon the addition of fertilizer phosphorus. **Maleina** (1958) reported that for phosphates to be well utilized by plants, large amounts of phosphorus fertilizer are required for acid soils while small amounts may be sufficient for neutral, carbonate and limed soils.

For a soil of pH 6.6, **Khanna—Mahajan** (1971) reported that conversion of the added KH_2PO_4 was in the form of $Al-PO_4$ (35 to 56%) and $Fe-PO_4$ (17 to 34%). In a soil of pH 8.3 (2.0% $CaCO_3$), on the other hand, saloid bonded-P and $Ca-PO_4$ were much more than in acid soil. In their experiments on the conversion of water-soluble phosphates to less soluble forms, **Laverty—McLean** (1960) studied 12 soils. The pH range was 4.7 to 7.3 and it was reported that as the pH increased, samples showed a tendency for more phosphate in the $Ca-PO_4$ fraction and less phosphate in the $Fe-PO_4$ fraction. Differences in the availability of the reaction products would partly account for the observed variations. **Olsen** *et al.* (1956) compared the availability of phosphate carriers to winter wheat as affected by the method of placement and nature of soil. It was reported that maximum effectiveness of the more soluble forms of phosphate fertilizers occurred on calcareous soils.

The "A" values (**Fried—Dean** 1952)* were high for the "pellet" method of fertilizing for all crops, but these differences were significant for flax and wheat only. The "A" values for rape and flax were similar in the "spread" treatment the values for flax were about twice as high as that for rape. Because the soil phosphorus uptake by flax (like other crops) was not different for the two methods, a very high "A" value for the "pellet" treatment of flax is due to its much lower uptake of the fertilizer when the crystals were placed in the centre of the pot than that from the crystals spread uniformly.

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* "Let us assume a soil with two sources (A and B) of a nutrient (phosphorus for example). If the plants growing in this soil absorb nutrient from the two sources in direct proportion to the respective amount available then:

$$\frac{A_{\text{soil}}}{B_{\text{soil}}} = \frac{A_{\text{plant}}}{B_{\text{plant}}} \tag{1}$$

Equation (1) provides a basis by which the available nutrient in a soil can be compared with that of a standard and its amount measured. Solving for B_{plant} in terms of y, and substituting

$$A = \frac{B/1 - y}{y} \tag{2}$$

Accordingly, the amount of available nutrient in the soil, A, can be measured if y, the proportion of nutrient in the plants derived from the standard can be determined."

However, certain factors such as the extent of root growth, the presence of other nutrients, etc. (KALRA 1966, 1971c) would affect phosphorus utilization from calcareous as well as non-calcareous soil. The initial level of available phosphorus and the phosphate fixing capacity are important in determining the response to fertilizer phosphorus (VOLK—McLEAN 1963). The soil used for the present experiment had higher amounts of available phosphorus as compared to the soils used in the previous experiments. The amount of soil phosphorus absorbed by the plants would be affected by the soil reaction. JOHN *et al.* (1967) observed that for lucerne the relationship between soil phosphorus and plant phosphorus differed, besides other factors, with the soil pH.

The availability of phosphorus from the fertilizer applied by a particular method is different for calcareous and non-calcareous soils. Many physical and chemical conditions inherent to soils which were used in earlier investigations with calcareous soils and the present experiment with non-calcareous soil would influence phosphorus utilization and thus be responsible for the observed variations. For further evaluation of these factors, similar experiments should be carried out in the field.

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PHOSPHORUS, LIPID AND PHOSPHOLIPID CONTENTS OF MYOFIBRILLAR PROTEINS

II. LIPID AND PHOSPHORUS CONTENT OF MYOSIN

By

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The paper deals with the heterogeneity of myosin and with its phosphorus and lipid content. Changes in the phosphorus content of myosin were found to depend on the method of preparation (3—4.5 g atom phosphorus per $5 \cdot 10^5$ g protein). The phosphorus content of chromatographed fractions also changes and in accordance with the conditions of separation settles at a concentration determined by the concentration of pyrophosphate. The lipid content of myosin was found to be much higher than the 4 per cent suggested by the literature (Lynn 1965). Owing to the aggregation of the giant molecule the whole lipid content can be removed from the myosin with Chl-MeOH only after a very long time and repeated treatments, but, when subjected to dialysis, lipid is slowly released from the molecule, the greater part of which appears in the dialysing water. Myosin from which a part of its lipid content has been removed gives an ultra violet spectra, and a difference extinction spectra can be drawn up, which cannot be done with crude myosin. On a DEAE-cellulose column the myosin can be separated at least into four fractions, but only fraction III shows a single component electrophoretically. Under the influence of dialysis the chromatographed fractions — when examined separately — release some more lipids, moreover it is only then that the closely bound lipid left behind can be obtained through extraction by a mixture of Chl : MeOH. When freshly isolated the lipids of myosin are colourless liquids, which in the air quickly autooxidize and become a brownish resin-like material polymerized in an aspecific way. On TLC sheets (silica gel) lipids released by dialysis show 6—7 spots, while chromatographed myosin fractions only 2—3 spots (at the places of lecithin and cephalin), which disappear after the autooxidation and show a different localization.

Introduction

In a previous publication (Fazekas *et al.* 1971) the authors described that from the chromatographic fractions of myosin a non-protein lipid fraction autooxidizing in the air can be removed, which may have a role in maintaining the structure of the myosin.

Namely, many authors have proved the heterogeneity of myosin without proteolytic enzymes being applied.

Kielley—Harrington (1960) found that in 5 M guanidin. HCl myosin is composed of at least three subunits. Small *et al.* (1962), Young *et al.* (1962) pointed out that in 12 M urea or 5 M guanidin. HCl the myosin molecule first spreads then dissociates into polypeptid chains. Lowey—Cohen (1961) supposed two, while Kielley—Harrington (1960), Young *et al.* (1962) three natural subunits. The molecular weight of large peptides was found to be

200 000 g by SMALL *et al.* (1962), and 215 000 g by DREIZEN *et al.* (1967). On an acrylamide gel of 2.6 per cent FLORINI—BRIVIO (1969) could even separate the large peptides of myosin by electrophoresis.

In addition to components of high molecular weight OPPENHEIMER *et al.* (1967), LOCKER—HAGYARD (1967a), LOCKER—HAGYARD (1967b), DREIZEN *et al.* (1967) demonstrated the existence of some 20 per cent, 20—30 000 low molecular weight proteins in myosin. PATERSON—STROHMAN (1970) found that the low molecular weight components are also released under the influence of dodecyl sulphate, and are identical with peptides released at 10.5 pH and correspond to components of 18 500—19 500, 32 100 and 33 000 molecular weight on acrylamide gel. With a comparative gel-electrophoretic method the light molecule components of chicken myosin were found by SARKAR—COOKE (1970) to be of 25 500, 17 600 and 15 200, while those of cattle myosin by SCOPES—PENNY (1971) to be of 22 500, 18 000 and 17 500 molecular weight.

TRAYER—PERRY (1966), GROSCHEL-STEWART (1971) consider the myosin to be an isoenzyme and think that this fact accounts for the high variation of myosin ATP-ase activity in the different muscle types.

PERRIE—PERRY (1970) found that light components are not required for the ATP-ase activity of myosin to develop. According to earlier literary data, the AMP deaminase (AMP-amino-hydrolase EC 3.5.4.6) is bound with a high adsorption energy on the surface of the myosin (SZÉKESSY-HERMANN—JOSEPOVITS 1949, SZÉKESSY-HERMANN—ZOMBORI 1954), as is choline esterase (acetylcholine esterase EC 3.1.1.7, VODNYÁNSZKY *et al.* 1961) the activity of which balances at a constant value during the usual myosin purification carried out by cyclic precipitation.

The idea of a connection possibly existing between the lipid and its subunits in the structure of the myosin molecule has been raised. After several years observation — in agreement with LYNN's (1965) results — the authors found that the easy removal of some lipids from the surface of the myosin molecule resulted in the increased ATP-ase activity of myosin; however, a part of the lipids could not be removed in the extraction experiments which led to the heterogeneity of myosin.

In the course of purification the authors followed the ATP-ase activity of the myosin fractions, the activity of the secondary enzymes, as well as their electrophoretic homogeneity and the changes in their phosphorus and lipid content.

The authors give an account of the fact that lipids released from the myosin fractions change as a consequence of autooxidation, and thus their position on the thin-layer silica gel slab changes too.

Material and Method

Myofibrils were obtained from back and leg muscles of four-month-old rabbits with the method of **Perry—Corsi** (1958). The myofibrillar myosin was covered with **Hasselbach—Schneider** (1951) solution. Both myosins were purified by cyclic precipitation. The myosin solutions were diluted with distilled water to a concentration of 0.025 M KCl; after precipitation and sedimentation the pure supernatant was decanted and discarded, the myosin centrifuged and suspended in 0.5 M KCl. The actomyosin was removed by diluting to 0.25 M and centrifuging. The myosin was precipitated by a repeated dilution and finally chromatographed on a DEAE-cellulose column with the technique of **Baril** *et al.* (1966), after adapting the method elaborated for chicken myosin to rabbit myosin. Out of some 10 chromatographic experiments elution solutions with the most adequate composition were selected and their optimum concentrations determined.

Myosin was obtained from ground muscles after **Szent-Györgyi** (1947), with the modified method of **Portzehl** *et al.* (1950), by repeated precipitation and chromatographic purification on DEAE-cellulose.* Preparations dialysed and dried at 105°C were used for analysis. Phosphate-free myosin — with the view of controlling the absolute phosphorus content of myosin — was obtained with the extraction method of **Finck** (1965) and purified by the $(NH_4)_2SO_4$-technique.

The protein concentration was determined by measuring the N content with the Kjeldahl method, or with the microbiuret method after **Goa** (1963), and the spectrophotometric method of **Young** (1967) respectively. The ultra violet spectra of the individual fractions was prepared with a Beckman (model G) spectrophotometer.

The lipids were extracted according to **Folch** *et al.* (1957), with a mixture of Chl : MeOH : water = 65 : 33 : 2 v/v/v from preparations dried in vacuum thermostat. The phosphorus content was determined according to **Fiske—Subbarow** (1925), but the last reduction (to a final volume of 1 ml/10 ml) was made by adding 1 per cent ascorbic acid according to **Lowry** *et al.* (1954). The lipids were classified by thin-layer chromatography on silica gel slabs, as described in detail in the authors' previous paper (**Fazekas** *et al.* 1972) cited under the chromatograms.

The homogeneity of the myosin fractions was examined by polyacrylamide gel electrophoresis, on 19×9×0.6 cm slabs, according to **Akroyd** *et al.* (1967). 4 per cent acrylamide gel slabs with 3 per cent cross-chain (10 g acrylamide, 0.3 g methylene-bisacryl-amide) were prepared in a buffer system containing 8 M urea and 1 per cent 2-mercapto-ethanol. When preparing the gel slabs a dentate plexy (perspex) bottom plate was used, and after the samples had been taken, the 5 mm deep and 6 mm wide brick-shaped groove, forming after the polymerization of the gel was covered with a 10—12 mm thick freshly made polyacryl-amide gel layer. The preparation of the gel and the buffer system, the staining, and removal of the superfluous paint were carried out similarly to the procedure followed by **Perrie—Perry** (1970), but the duration of the electrophoresis was more than 4 hours. At the beginning the electrophoresis took place at a current flux of four, then eight mA/cm^2 gel, at room temperature, and during this 16—18°C water flowed through the cooling system of the apparatus. Under such conditions the chromatographic fractions enter the gel, only the non-chromatographed control in the pyrophosphate buffer, and a considerable part of fractions I, and Ia remain at the starting lines.

Results

Phosphorus and lipid content of myosin. The total lipid content of crude myosin isolated from ground muscles is highly varying. When the aqueous solution of myosin preparations is used as initial material 13—33

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* DEAE-cellulose DE 32 (capacity — 1.0 mequiv/g) was purchased from Whatman, Sephadex G-200 — from Pharmacia, Uppsala, TRIS-(hydroxymethyl)-aminomethane — from Calbiochem, ATP (Adenosine-5'-triphosphate), Urea, Natrium pyrophosphate and other chemicals from Reanal, Budapest.

per cent total lipid can be extracted. After repeated precipitation fluctuation in the lipid content is negligible. After centrifuging the solution for 2 hours at 105 000 g and 0°C, and removing the supernatant lipid, a perfectly transparent myosin solution is obtained. Its lipid content is much lower than that of the crude myosin. Without ultracentrifuging the myosin remains opaque even after precipitation. Myosin obtained by chromatographic separation, from DEAE-cellulose with KCl-gradient has a varying lipid content; 6—8 per cent when starting from wet gel and 6 per cent when from preparations dried at a temperature of 105°C. Dry myosin can no longer be dissolved; it is a swelling rubber-like elastic mass which can hardly be crushed in a knife-homogenizator, and even when homogenized for 2 hours in 20 per cent methanol shows hardly any change and gives a rough suspension only. The first extract is obtained by adding two unit volumes of chloroform, homogenizing for 20 minutes and extracting for one day. Further extracts are obtained by adding 10 vol extracting solution and extracting for 1—2 days. Both myosin purified by repeated precipitation and that chromatographed with linear gradient KCl are electrophoretically heterogeneous containing further substances of non-protein nature, therefore this method is not suitable for removing them.

Myosin obtained by the FINCK (1965) method passes through many phases of isolation and has, accordingly, a lower total phosphorus content of 3.1—4.1 M P/10^5 g. The amount of extractable lipid is 2—3 per cent which contains 0.4—1.1 M phosphorus. The myofibrillar myosin contains 4.5—5.0 g atom phosphorus per 5×10^5 g protein, and its total lipid content isolable by lipid solvents is 6—8 per cent. Extracts II and III contain small quantities of biuret positive substances which — after the lipid solvent has evaporated and re-dissolved — precipitates and can be removed by centrifuging. The lipid, phospholipid and phosphorus content of various myosins are summed up in Table 4.

Further experiments suggested that the data did not represent the total lipid content of the myosins summarized in the table. When comparing them with the gravimetric examinations of simple proteins it was found that in the case of myosin variable results were obtained for the phosphorus content, gravimetric weight and amount of substances removed by extraction. Discrepancy shown by the variability of these data is highly significant. Variability was thought to be in relation with the heterogeneity or structure of the subunits of the myosin. As to heterogeneity the following observations were made.

In the course of cyclic precipitation some biuret negative component was always left behind in the supernatant, though the major part of the lipids precipitated with the myosin. The lipids causing the opalescence of myosin can be removed by ultracentrifuging, but when dialysed against distilled water it again releases low molecular weight components.* From myosin dialysed against a 0.5 M KCl solution separation of more than one low molecular weight component was observed.

The lipid fraction recovered from the dialysing solution by extraction and concentrated is a yellow liquid which later becomes a resin-like mass as a result of autooxidation.

From ultracentrifuged myosin after repeated extractions at 0°C with a mixture of chloroform and methanol (2 : 1, v/v), then Chl : Me v/v, and finally pure methanol in some six months a totally lipid-free protein preparation could be obtained that did not go grey in the air; at the end of the isolation the protein value determined from the Kjeldahl N-content of the initial material (881 mg) corresponded to the gravimetric value of myosin (884 mg). The gravimetric value of the substances extracted and collected during this period was 320 mg (38 per cent) after concentration. Owing to what has been said above, only methods by which myosin can be separated into its subunits and sufficient amounts of material obtained for analysis are considered suitable for the determination of the lipid and phosphorus content of myosin.

Table 1

*Total lipid, phospholipid and phosphorus content of myosins isolated by different methods**

Myosin	Number of isolations	Isolable lipid/weight-%	Phosphorus content		
			before extr. g atom P/10^5 g	lipid phosphor g atom P/10^5 g	protein fraction g atom P/M myosin
Isolated from ground muscle:					
by a single precipitation	3	8—16	5 —7.5	2 —4	3.5—4.5
chromatogr. dry gel	1	6	6.7	2.6	4.1
chromatogr. wet gel	2	6—8	4.0—5.5	0.9—1.5	3.1—4.0
chromatogr. repeated precipitation	2	2—3	3.8—4.5	0.8—1.3	3.0—3.5
Finck	2	2—3	3.5—4.0	0.4—1.1	3.1—4.0
myofibril	3	6—8	4.5—5.0	1.5—2.0	3.1—4.5

* The adsorbed P content is not involved.

The authors obtained good results during previous studies (FAZEKAS *et al.* 1971) with the method of BARIL *et al.* (1966) mentioned in the methodological part of this paper; after some modification myosin can be separated by this method into 4—5 protein fractions and at least 2 lipid fractions. Table 1 shows the myosin fractions separated by this method and presents the eluents used.

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* Dialysis was always carried out by using dialysing membranes and strings boiled in 5 per cent $NaHCO_3$ solution and washed out in distilled water.

Table 2

Distribution of myosin fractions from rabbit skeletal muscle when chromatographed on DEAE-cellulose column

Fractions	E_{280}		%	E_{280}/E_{260}
I	1— 56	84	25.7	1.68
Ia	57—102	37	11.3	1.53
II	103—146	45.06	13.8	1.42
III	156—168	35.95	11.0	1.80
IV	201—246	65.83	20.2	1.44
V	249—260	4.74	1.45	1.04
VI	261—283	23.82	7.65	0.74
VII	284—291	15.31	4.65	0.70
Recovery		311.8	95.6	
On the column		327.8	100.0	1.28

Table 2 shows the percentage distribution of chromatographic myosin fractions, the percentage recovery, and the quotients E_{280}/E_{260}.

The value of the percentage recovery of the myosin fractions proves the correct selection of the ion exchanging and elution solutions. Fig. 1 shows that the curve of fractions V and VI drawn on the basis of optical density measured at 260 millimicron is reversed, and is much higher than the value of E_{280}. Fractions V and VI are biuret negative, optically turbid, and their amounts range between 13 and 20 per cent in the different preparations. These substances are mainly of a lipid nature, made impure by some RNA. As they did not strictly belong to the myosin molecules and could be separated from them on an ion exchanging column, they were not subjected to thorougher examination. If prior to chromatography the myosin was gel filtrated on a Sephadex G 200 column, the fractions V and VI were considerably reduced.

The other fractions are proteins. The proportions of the individual fractions seen in the figure vary to some extent from preparation to preparation, thus showing that the classical myosin is heterogeneous. All fractions display adenosine-triphosphatase activity, and — with the exception of fraction III — some cholinesterase and AMP-deaminase activity of varying intensity. Table 3 presents the enzyme activities of the fractions.

The data included in Table 3 are the mean values of the enzyme activities measured in five preparations. In many cases fraction Ia is small, and a large proportion of cholinesterase and AMP-deaminase activity is found in fraction I. It should be mentioned here that fraction IV appeared in some cases

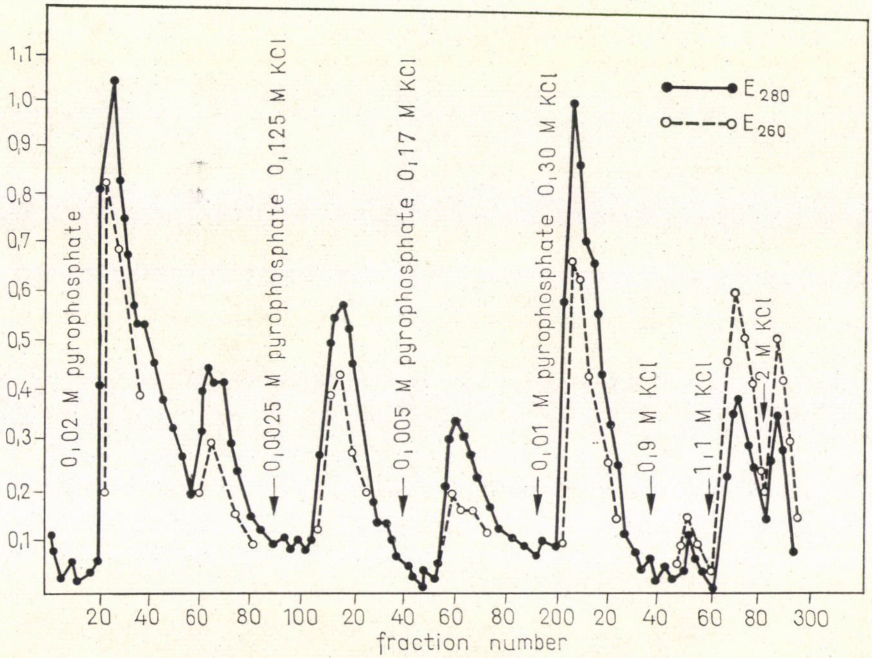


Fig. 1. Chromatography of the ultracentrifuged and pyrophosphate equilibrated myosin on DEAE-cellulose column. 6.2 ml fractions collected

Table 3

Enzyme activity in the different fractions

Enzyme activity	Fractions							
	0	I	Ia	II	III	IV	V	VI
ATP-ase* μmol Pi/mg/min.	0.15—0.2	0.07	0.07	0.20	0.31	0.26	—	—
Cholinesterase*** μg ac ch/mg/h	0.48	58	120	44	0	22	—	—
AMP-deaminase** μg N/mg/h	1237	492	1289	400	0	60	20	—

* Holland—Perry (1969)
** Hestrin (1949)
*** Nessler — NH_3 determination after Ács—Hermann (1949)

as three not readily separating peaks, and the ATP-ase activity of the peaks was 0.30, 0.24 and 0.32 respectively.

In the course of purification the E_{280}/E_{260} quotient of the individual fractions measured at pH 7.2 generally increases, while in the case of the non-protein fractions of V and VI decreases, as seen in Table 4.

Table 4

Quotient of E_{280}/E_{260} in fractions separated on DEAE-cellulose column

Number of experimental animals	Before separation 0 fraction	I	Ia	II	III	IV	V	VI
130	1.18	1.56	—	1.87	1.87	1.48	0.86	0.77
131	1.12	1.48	1.53	1.42	1.77	1.44	0.80	0.70
132	1.11	1.26	—	1.27	1.76	1.44	0.70	0.64
134	1.28	0.89	1.29	1.48	2.02	1.69	0.68	0.58

Table 5

E_{280} values of fraction 0 and the separated fraction by mg/ml concentration (pH 7)

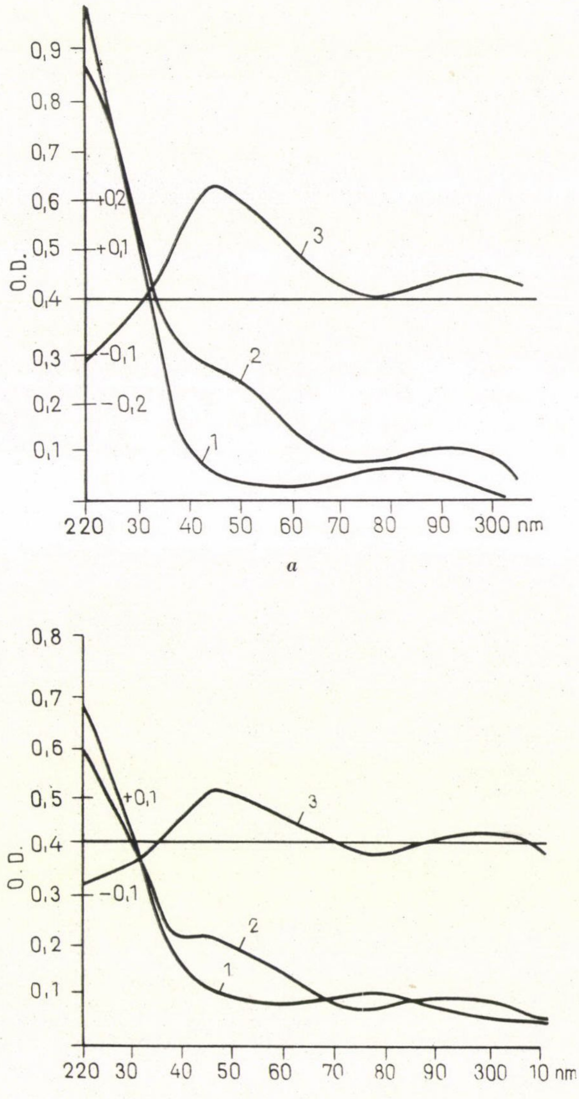
No.	Fractions					
	0	I	Ia	II	III	IV
130	0.572	0.700	0.515	0.593	0.590	0.582
131	—	0.600	0.430	0.638	0.585	0.570
132	0.540	0.497	0.250	0.636	0.565	0.632
134	0.585	0.712	0.300	0.628	0.580	0.601

Fractions I—IV have characteristic UV spectra, Fig. 2(*a-f*) and difference extinction spectra. It is known that myosin has a characterless, flat spectrum, which in the course of purification by cyclic precipitation becomes more and more characteristic of proteins (LOWEY 1965). That is why it seems worth presenting the spectra and difference extinction spectra of fractions with 0.5 M present.

According to YOUNG (1967) the absorption value at 280 nm characteristic of myosin concentration is = 0.540 ml/mg (pH 7.0). This value was found to be $\varepsilon = 0.56$ ml/mg (7.0 pH) by SMALLER—FINEBERG (1964), $\varepsilon = 0.572$ $E_{278}—E_{320}$ ml/mg (pH 7.0) and $\varepsilon = 0.636$ $E_{290}—E_{340}$ ml/mg (pH 13) by MIHÁLYI—ROWE (1966) respectively. With the authors' preparations this value ($\varepsilon = 0.540—0.585$ E_{280} ml/mg) is characteristic mostly of fraction 0 before the chromatography by the DEAE column, while in the separated fractions highly differs from this, as seen in Table 5.

The authors cited deducted absorption originating from light dispersion from the characteristic values; in the experiments reported in this paper this was negligible.

Homogeneity examinations of myosin fractions were performed with polyacrylamide gel used in a way described in the methodological part of the paper. Fig. 3 shows the non-chromatographed control fraction balanced



a

b

in pyrophosphate buffer, and fractions II, III and IV. The concentrated fraction of I, and fraction Ia — not shown by the figure — are highly heterogeneous suggesting the presence of at least four or five proteins.

The number of proteins suggested electrophoretically to be present in the various fractions are: 4 in the control, 2 in fraction II,* 3 in fraction IV, while only 1 in fraction III. The control fraction and the greatest part of the

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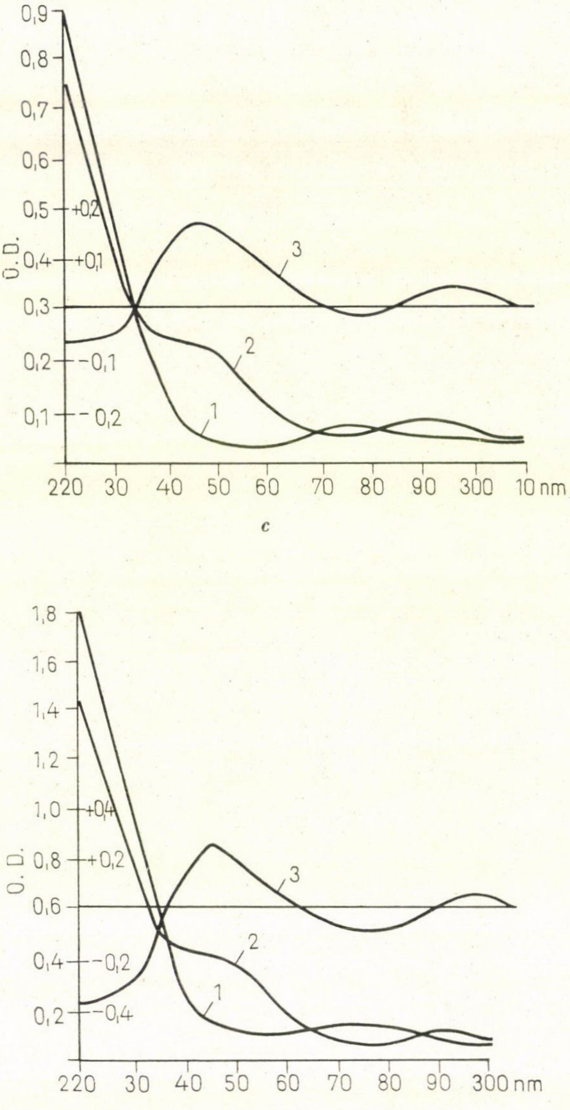
* mostly is single one

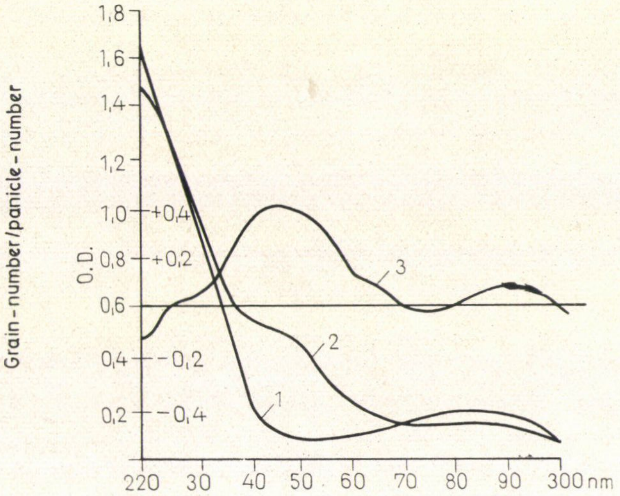


c

d

ultracentrifuged myosin fraction do not enter the gel. On the basis of spectrophotometric measurements fractions V and VI originating from myosin, amount to 12—20 per cent, but gravimetric measurement shows higher values than that.

The protein fractions were subsequently dialysed against 0.5 M KCl solution, and the low molecular weight substances measured spectrophotometrically. After the third change the absorption of the substances found in the dialysing solution was insignificant, therefore the protein fractions were



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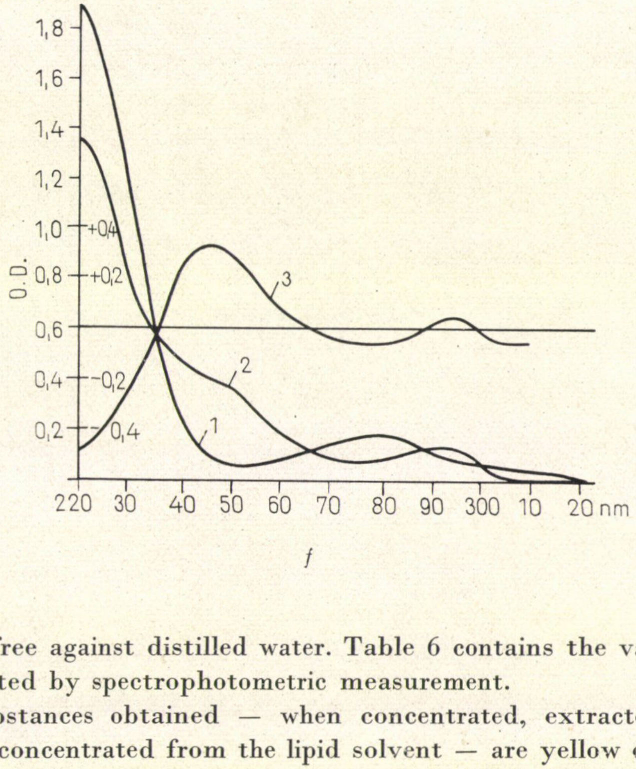

f

dialysed ion-free against distilled water. Table 6 contains the values of substances detected by spectrophotometric measurement.

The substances obtained — when concentrated, extracted from the solution and concentrated from the lipid solvent — are yellow oily residues.

Fractions dialysed ion-free do not, supposedly, release further low molecular weight substances. Therefore the aqueous solutions of protein frac-

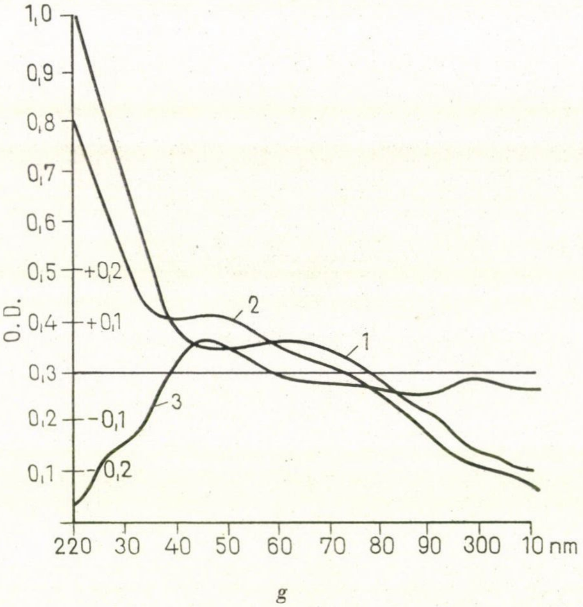

Fig. 2. Spectra and difference extinction spectra of myosin fractions from DEAE-cellulose column

Table 6

E_{280} value of low-molecular weight substances released from chromatographed fractions, as related to 100 g protein*

No.	I	Ia	II	III	IV	V
130	100 : 120	100 : 40	100 : 66	100 : 41	100 : 19	100 : 88
131	100 : 50	—	100 : 50	100 : 35	100 : 25	—

* The E_{280} value means no concentration and cannot be expressed in mg value due to the incomplete autooxidation.

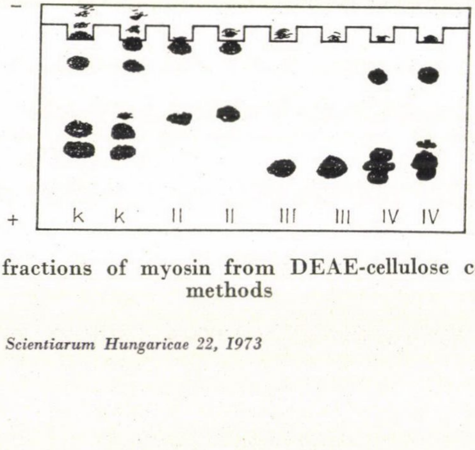

Fig. 3. Electrophoretic fractions of myosin from DEAE-cellulose column. Legend in the methods

tions were extracted three times with a mixture of Chl : MeOH (2 : 1) and concentrated. Residues thus obtained are considered closely bound lipids. The extracted protein fractions and the light yellow oil left behind were subjected to gravimetric measurement. The results are contained in Table 7.

The lipid obtained from fractions I—IV is only half a drop of light yellow oil. The question has arisen whether it is not the impurities of the lipid solvent that are concentrated during the preparation. The problem can be decided by the following: 1. The use of any kind of lubricant has been avoided in the experiments for years; 2. when concentrating the lipid solvents employed no residue was obtained; 3. the substances obtained were checked at the Institute of Organic Chemistry of the Eötvös Loránd University by an IR spectrophotometer against chloroform, and the Vmax (cm^{-1}) values were found identical with a slight deviation, as described in the case of actine (Fazekas *et al.* 1972).

Table 7

Ratio of protein to lipid in dialysed and ion-free myosin fractions
mg protein : mg lipid

No.	Fractions				
	0	I	II	III	IV
	P : L	P : L	P : L	P : L	P : L
131		75 : 25	85 : 15	86 : 14	87 : 13
132	888 : 318	50.8 : 50	65 : 35	91 : 9	78.4 : 21

After a gravimetric determination the protein residues — supposedly lipid-free — were reduced to ashes and their phosphorus content determined. The results are shown in Table 8.

The data show that the control contains a relatively steady amount of phosphorus. The phosphorus content in fractions II and III is not changed essentially by either chromatography, or dialysis or extraction. All fractions have higher phosphorus contents than myosin of any other origin, or produced by any method described in Table 1. The myosin and its fractions obtained through chromatography absorb phosphorus from the pyrophosphate buffer applied in the procedure, which cannot be removed either by purification or by any other technique, only adjusted to a characteristic concentration.

Lipids obtained by dialysis from ultracentrifuged myosin can be separated on silica gel slabs into six or seven fractions at least (Fig. 4).

The lipids of chromatographed myosins display two or three fractions and even more in an oxidated state when their localization on the silica gel slab also changes (Figs 5, 6).

Table 8

Phosphorus content of lipid-free myosin fractions, g atom Pi/5×10⁵ g protein

No	Fractions					
	0*	I	Ia	II	III	IV
130	8.8	8.3	—	11.4	8.88	16.8
131	8.8	1.5	8.7	11.6	8.65	9.9
132	9.2	2.0	—	10.5	9.22	10.99

* Fraction 0 means the phosphorus content of the control prior to chromatographic separation.

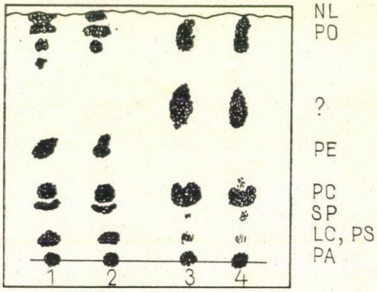

Fig. 4. Thin-layer chromatography of lipids of ultracentrifugated and dialysed myosin on silica gel plate by CUZNER-DAVISON (1966). Developed with Chl–MeOH–28% NH_3 (17 : 7 : 1, v/v/v). 1.2 lipid fractions immediately isolated, 3.4 lipid fractions after partial autooxidation. PA phosphatic acid, LC lysophosphatydil choline, Ps phosphatydil serine, SP sphingomyelin, PC phosphatydil choline, PE phosphatydil ethanolamine, PO peroxidized lipid, NL neutral lipid, partially oxidized lipid

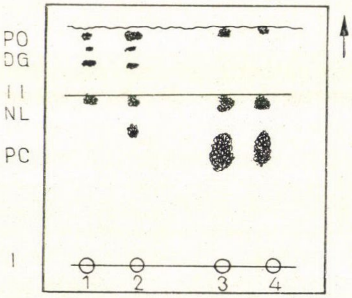

Fig. 5. Thin-layer chromatography of lipids from chromatographed myosin, II, III fractions by SKIPSKI *et al.* (1963). Developed with I. Chl–MeOH–acetic acid–water (50 : 25 : 7 : 3, v/v/v/v), II. n-hexane–diethylether–acetic acid (90 : 50 : 5 v/v/v). Latter as Fig. 4

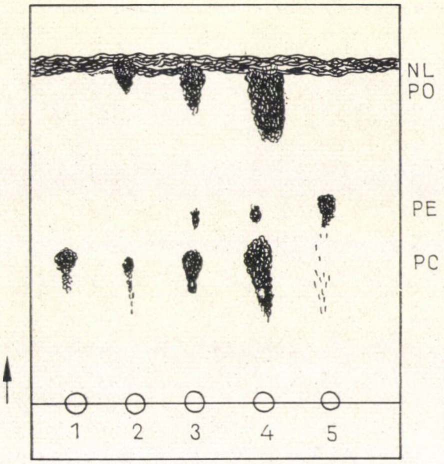

Fig. 6. Chromatography of lipids from chromatographed myosin by Wagner *et al.* (1961). Developed with Chl–MeOH–water (6.5 : 2.5 : 0.4, by volume). Detected by ester-bound reaction followed by spraying 10% phosphomolybdenic acid reagent or iodine vapour. 1 PC control, 2, 3, 4 lipids from myosin in turn about 25, 55, 100 μg, 5 PE control

Discussion

Efforts had been made earlier to determine the phosphorus content of myosin. Lajtha (1948) obtained a less than 1 g atom P/M result with actomyosin. Brahms—Rzysko (1959) found that in the rabbit heavy meromyosin anorganic phosphorus was present which in the course of ATP splitting increased and reached a maximum. Szörényi (1951) and Weber—Hasselbach (1954) also started investigations in the subject. On the ground of their results they assumed the existence of a phosphorilated intermediate and research was started to find it. Gergely—Maruyama (1960) found more than 1 g atom anorganic phosphorus, and in the presence of 10 mM ATP as much as 5 g atom phosphorus in 10^6 g myosin, while Kinoshita *et al.* (1969) even determined a 10—20 g atom phosphorus content after the enzyme activity. Both orthophosphate and ADP and ATP binding were observed in myosin. In this context exhaustive summarization is given by Dreizen—Gersman (1970). It can be seen that the concept of the absolute phosphorus content in myosin is interwoven with the phosphorus content released during the enzyme activity. Preparations with the least possible phosphorus content can be obtained by chromatographic purification and subsequent dialysation against 0.5 M KCl. Finck's myosin shows minimum phosphorus content even without chromatography. Data included in Table 4 and 8 are meant to show the phosphorus content developed as a function of the conditions of preparation, while myosin fractions separated on the DEAE-cellulose to show further phosphorus surpluses bound from the pyrophosphate buffer. NAUS *et al.* (1969) found that in a Tris-HCl buffer myosin binds the pyrophosphate the best, while in a phosphate buffer it is more or less driven out depending on the phosphate concentration, and at a phosphate concentration of 50 mM, at pH 7.0 hardly any pyrophosphate is bound. Data do not give information about the way how phosphate is bound.

We think, the actual phosphorus content of myosin depends not only on the nucleotide content, but also on the phosphate-ester forming protein kinase activity. PERRIE—SMILLIE—PERRY (1972) found that the one of the myosin light component (ML_2) is such a phosphorylated protein.

The lipid content of myosin was found to be 3—4 per cent by LYNN (1965) who noticed that its removal increased the stability of myosin. According to the authors' experiments the lipid content of myosin is much higher than this, and by a single extraction only lipids loosely linked with the myosin can be removed. In the course of purification by cyclic precipitation the lipids precipitate together with the myosin. Dialysis is considered a better method for removing the lipids, as under its influence that part of the lipids is released which takes part in forming the aggregate.

The amount of biuret negative components released in the dialysing solution can be followed by photometry and recovered by extraction. Unfortunately, even so, owing to the autooxidation the method is only suitable for obtaining a qualitative picture. The myosin of which the lipid content has been reduced by ultracentrifuging and dialysing can be separated on a DEAE-cellulose column into at least six fractions. The measuring of the enzyme activity of the fractions and its electrophoretic control show that only one or two fractions can be found that are homogeneous both enzymatically and electrophoretically. 33 per cent of the ATP-ase activity is contained in fraction III, 25 per cent in fraction IV, 12 per cent in fraction I, 5 per cent in fraction Ia, and the rest is distributed among the other fractions.

Fractions V and VI are not worth too much mentioning. When gel filtrated on a Sephadex G 200 column before being separated on the DEAE-cellulose column the two fractions are considerably reduced. Both fractions contain much fatty acid.

Fractions obtained by chromatography release lipids during the KCl dialysis, moreover, in the dialysed fractions further lipid contents were found, a little light yellow oil isolated. This small amount of lipid is worth being subjected to further examination, since it is probable that myosin has individual lipid content well distinguished from that of myofibrils.

Examinations concerning the myosin suggest a two-sided heterogeneity, from the point of view of proteins on the one hand, and lipid content, on the other, inasmuch as the small amount of lipid present may take part in developing the structure of myosin.

In the authors' experiments an explanation is found to Lowey's (1965) observations according to which myosin purified by a single precipitation gives no difference spectrum, while after repeated precipitations gives a spectrum characteristic of proteins, from which a difference spectrum can be obtained.

Acknowledgement

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PROTON MAGNETIC RESONANCE STUDIES ON VEGETABLE OILS AND SEEDS

By

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Proton magnetic resonance was measured in seeds and extracted oils of three oil plants by means of a wide-line nuclear magnetic resonance (NMR) spectrometer. NMR spectroscopy is able to separate the NMR spectrum of protons in solids (e.g. cellulose) and that in liquid — or solid fats. The apparatus is sensitive enough to perform measurement on a single seed with sufficiently high signal-to-noise ratio. Free water content does not perturb the measurements of the oil content.

Introduction

Application of the proton NMR (hereafter PMR) measuring technique in agricultural research and plant breeding has already been reported (Tolnay 1971). The present paper wishes to show how the wide-line NMR apparatus, constructed to study the physical problems of solids in the Central Research Institute for Physics, can be used for the quantitative determination of oil content in intact plant seeds. We wanted to see whether the resolving power of the apparatus is sufficient to distinguish protons in different chemical environments, and whether the sensitivity of the apparatus is sufficient to study a single seed.

The physical principles of PMR are well-known. The works cited (Lösche 1957, Kittel 1966) provide sufficient information. Organic chemical application of this technique was reported by Hopkins—Bernstein (1959). Water content was determined by Shaw (1950) measuring PMR signal. Again Hopkins (1961) gave account of his determining the oil contents of seeds by using a wide-line NMR apparatus. Determining the oil contents of intact seeds Conway—Earle (1963) dried the seeds for fifty hours at 50°C. It was after drying that they took the PMR spectra of oils. The total oil content was calculated from the spectra. The shape of the PMR signal obtained depends on the composition and saturation of the oil, and on the amount of oxygenized acids. The authors computed the hydrogen contents of the individual vegetable oils. The calculated and measured values are in agreement. In Hungary this type of PMR signal was detected on a maize grain, first in 1968 (Tompa 1968).

Material and Method

The wide-line NMR apparatus used was developed in the Laboratory of Solid State Physics at the Central Research Institute for Physics to study the special problems of solids. A detailed description of the apparatus can be found in a paper of TOMPA—TÓTH (1963). The present measurements were done in a magnetic field of 4500 Gauss, the modulation field intensity was between 0.4 Gauss, and 1.0 Gauss.

Plant samples were placed into a glass tube of 10 mm in diameter and put into the detector. The diameter of the detector's sensitive volume was 12 mm, while its length 15 mm. The samples were marked as follows: 1/a sunflower cooking oil; 1/b rape cooking oil; 1/c soybean oil extracted with petroleum ether; 2/a sunflower seed (air-dry); 2/b rape seed (air-dry); 2/c soybean (air-dry); 3/a a single seed of sunflower (dried); 3/b oil-free groats of sunflower seeds.

Drying was carried out in a vacuum desiccator at 50 °C over 72 hours. Measurements were performed at 20 °C.

Results

PMR curves obtained on vegetable oil extracts and seeds are presented in Figs 1, 2 and 3. The curves are the first derivates of the PMR signal. The width of the absorption PMR signal is defined as the distance of peaks on the derivative signal.

Fig. 1. Derivative of the PMR signal with respect to the magnetic field intensity, in sunflower oil (a), rape oil (b), soybean oil extracted with petroleum ether (c). Modulation field intensity 0.4 Gauss

Discussion

Spectra seen in Fig. 1 unequivocally show that in the cases of 1/b and 1/c the PMR spectrum is formed by two PMR signals superposed on each other. The wider (1.5 Gauss) PMR signal was superponed by the narrower (0.5 Gauss) PMR signal. The two derivative spectra cut the base line in one

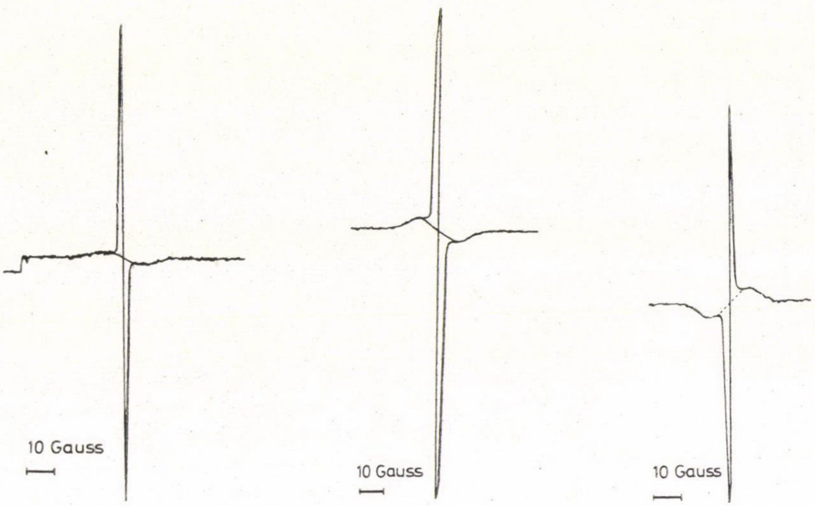

Fig. 2. Derivative of the PMR signal with respect to the magnetic field intensity, in sunflower seed (a), rape seed (b) and soybean (c). Modulation field intensity 1.0 Gauss

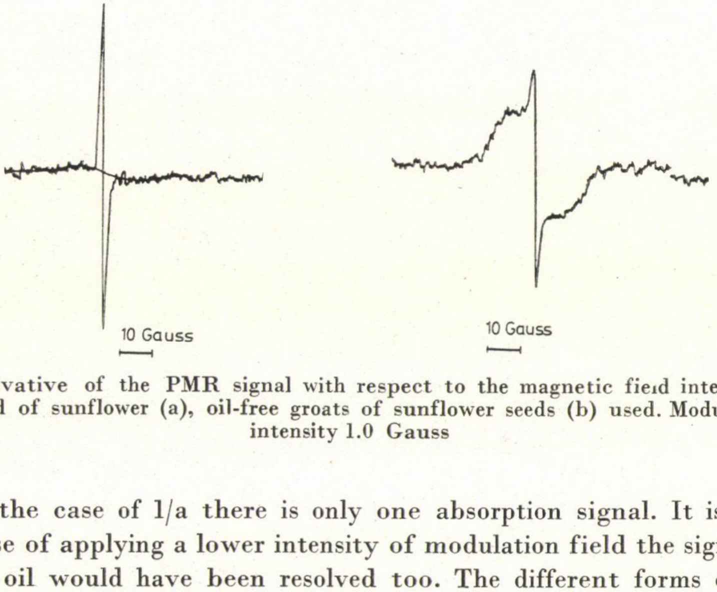

Fig. 3. Derivative of the PMR signal with respect to the magnetic field intensity, with a single seed of sunflower (a), oil-free groats of sunflower seeds (b) used. Modulation field intensity 1.0 Gauss

point. In the case of 1/a there is only one absorption signal. It is possible that in case of applying a lower intensity of modulation field the signal of the sunflower oil would have been resolved too. The different forms of signals can be traced back to differences in fatty acid composition between the various oils.

Curves in Fig. 2 show that protons in oils (the width of the signal is 4.5 Gauss) and those in other substances, e.g. cellulose (the width of the signal is 18 Gauss) have essentially different chemical environments. As a conclusion the detection of the PMR signals is an adequate method to determine the oil content of intact plant seeds.

Fig. 3/b shows that the narrower (4.5 Gauss) component of curves seen in Fig. 2 really originates from protons in the oil. It proves, besides, that the extraction is not of 100 per cent.

Fig. 3/a suggests that the apparatus is suitable to detect the NMR signals of protons in a single seed, at a signal/noise ratio of 14/1.

It must be emphasized — though Figs 1 and 2 clearly show — that the correct determination of the intensity of the modulation field is an essential aspect of measuring technics. The width of signal is partly a function of the modulation field intensity. It is desirable to perform preliminary measurements to determine the most favourable value of field intensity, at which signal/noise ratio and resolving power are optimal. Low intensity modulation field improves the resolution, the narrow oil lines superposed to the wide line of cellulose become apparent, while the line of the cellulose occasionally disappears. In case of a high intensity modulation field the signal of the cellulose is clearly seen, but the PMR signal originating from the oil disappears.

The fact that no difference was found between the signal (2/a) of air-dry seeds (10—12 per cent moisture content) and the signal (3/a) of dried seeds proves that with the resolving power applied free water content does not affect the measuring of the oil content. This was to be expected, as the PMR signal of water protons is narrower by several orders of magnitude than the signals detected by us. The latter proves that in the natural state of the seed measuring gives information on the oil content.

Acknowledgement

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SPECIES OF THE BRASSICA GENUS DISTINGUISHED BY THE PHOTOMETRIC STUDY OF THE COLOUR SUBSTANCE COMPLEX IN THE SEED

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In the *Brassica* genus the colour substance complex of the seeds varies from species to species. In a 1.5 molal solution of NaOH the colour substance dissolved with various colours. There was no difference in the colour of fluorescence between the solutions and the seeds soaked, but the colour of the solutions was different when looked at in daylight. The colour of the solutions could be distinguished even more accurately on curves prepared by spectrophotometry. On the basis of the colours of the solutions and the curves the species and varieties studied can be classified into groups. On the basis of the colours and spectrophotometric curves of the solutions mentioned the species and varieties in the different groups can be readily, while within a group approximately identified.

Introduction

Seeds in the *Brassica* genus are difficult to distinguish by traditional morphological (Berggren 1962, Musil 1948, Hofsten 1970) and cytological (Sikka 1940) methods. Some species have identical chromosome numbers, therefore the species can only be identified by the morphological study of the somatic chromosomes (Sikka 1940). The light absorption of the solution made of the colour substance of the seed with benzene and methanol showed differences between 460 and 700 millimicrons making *B. oleracea* v. *capitata* f. *alba* and f. *rubra* distinguishable. Fluorescence of the extract was studied too (Schuphan 1948). With similar methods, on the basis of the solution of carotinoides Schuphan (1949) was able to separate the green and lilac coloured forms of *B. oleracea* v. *gongyloides* (caulorapa) too. Studies by Bate-Smith (1948) and Weinman (1956) were build on the leuco-anthocyanin of the seed coat and colour substances bound to the plastides. Vaughan—Denford (1968) separated the *Brassica* and *Sinapis* species by acrylamide gel electrophoresis.

The investigations mentioned raise the question of species differing from each other in the colour substance of the seed. Colour substances were expected to dissolve in a proper solvent with various colours. The investigations were aimed at finding the proper solvents and elaborating a simple method by which the seeds can be identified.

Material and Method

The starting experiments were aimed at finding the proper solvent. Chemicals tested were: KOH, NH_4OH, $Ca(OH)_2$, NaOH, H_2O, NaH_2PO_4, $NaNO_3$, $NaClO_3$, methanol. Concentrations used were: 0.1—1.0—1.5 gram molecule solutions. The material studied was:

1. *Brassica oleracea* L. convar. *acephala* (DC) Alef., field kale;
2. *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *capitata* (L.) Alef. f. *rubra* (L.) Thell., red cabbage;
3. *Brassica oleracea* L. convar. *capitata* (L.) Alef. f. *alba* DC., common white cabbage;
4. *Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *sabauda* L. (syn. var. *bullata* DC.), savoy;
5. *Brassica oleracea* L. convar. *acephala* (DC) Alef. var. *gongyloides* L. (syn. *B. caulorapa* DC), kohlrabi;
6. *Brassica oleracea* L. convar. *oleracea* L. var. *gemmifera* DC., Brussels sprouts;
7. *Brassica pekinensis* (Lour.) Rupr., Chinese cabbage;
8. *Brassica rapa* (L.) Metzg. ssp. *rapifera* (Metzg.) Alef., turnip;
9. *Brassica napus* (L.) Metzg. var. *napus* f. *annua* (Schübl. et Mart.) Thell., rape, — spring —;
10. *Brassica napus* (L) Metzg. var. *napus* f. *biennis* (Schübl. et Mart.) Thell., rape, — autumn —;
11. *Brassica nigra* (L.) Koch., black mustard;
12. *Brassica juncea* (L.) Czern. et Coss., brown mustard;
13. *Brassica rapa* L. var. *silvestris* (Lam.) Briggs. f. *campestris* L., late turnip;
14. *Brassica carinata* A. Braun, Abyssinian cabbage.

Varieties studied:

2. Holland export, Langedijnen Winter, Dansk export, Früh dänisch Hunderup P 62, Dauerhaft Amager Tofto S 60;
3. Ditmarscher extra früher, Cottage Prise Drumhead, Amagerhof;
4. Vertus, Eisenkopf Wirsingkohl;
5. Prague Market White, Knudekohl, Wiener Weisser Treib, Wiener blauer Treib;
6. Früh Zwerg Tofto P 60, Hercules, Focus F 1., Amager, Polarsterjnen Hunderup P 65;
7. Chilii;
9. Target, Gyllen, Regina;
10. Allie, Panter, Vestal, Victor, C.I.V. The seeds were obtained from the Statsfrøkontrolle, Copenhagen and from the Seed Producing and Supplying Enterprise, Budapest.

A glass tube of 3 in mm diameter and 0.3 cm^3 solution were used for the study of each seed. Fluorescence of the solution after it had dried was examined on a non-fluorescent filter paper with an Osram I. 20 W/73 type UV fluorescent tube.

Photometric determination was carried out using 3 ml solution and 15 seeds. Light absorption was measured with a Beckman DB-G Grating spectrophotometer, and the absorption curve was prepared immediately by a W + W electronic 2211 Recorder attached to the apparatus. The examination was performed in Taastrup (Denmark). The tests were repeated in Budapest with a MOM spectrophotometer. Each measuring was repeated four or five times.

Colouration of the solutions began after one and a half hours, and the characteristic colour developed in three hours. Colours became dark after 24—48 hours. The colours were compared to the range of colours in the Colour Chart (London). The investigation did not include the chemical analysis of the coloured solutions obtained.

Results

1. *Study on the fluorescence of solutions.* The described solutions dissolved fluorescent substances from the seeds. The solution absorbed by the filter paper — after having dried — fluoresced in the blue colour. In the 1.5 molal solution of NaOH, exceptionally, a yellowish green colour was produced by the crushed seeds, the cause of which was not investigated.

The intensity of fluorescence varied according to the concentration of the solutions. Time (2 months) did not affect the fluorescence of the papers.

2. ***Study of coloured solutions in natural light.*** The colour substance complex of the seeds dissolved the most perfectly and with the most divergent colours in the 1.5 gram molecule solution of NaOH. Attempts were made to compare the colours of solutions with those of the Colour Chart (London, 1966). The colour of a liquid can be compared to the solid colours of the colour chart only approximately. Results obtained from the comparison of colours after a 4-hour standing of the solutions:

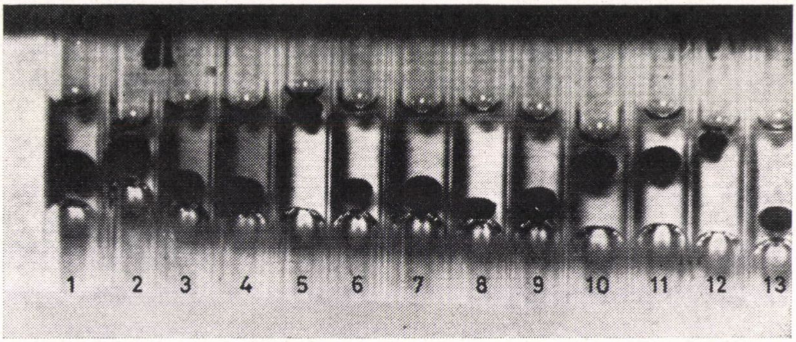


Fig. 1. 3 mm glass tubes containing various colour seed extracts in 0.3 ml 1.5 gram molecule NaOH. Species and varieties studied: 1. *Brassica oleracea* cv. *acephala;* 2. *B. oleracea* v. *capitata* f. *rubra;* 3. *B. oleracea* cv. *capitata* f. *alba;* 4. *B. oleracea* v. *gemmifera;* 5. *B. pekinensis;* 6. *B. oleracea* v. *sabauda;* 7. *B. oleracea* v. *gongyloides;* 8. *B. rapa* ssp. *rapifera;* 9. *B. napus* f. *annua;* 10. *B. napus* f. *biennis;* 11. *B. nigra;* 12. *B. juncea;* 13. *B. rapa* ssp. *campestris*

1. *Brassica oleracea* cv. *acephala*	yellow orange	17/c
2. *Brassica oleracea* v. *capitata* f. *rubra*	yellow orange	14/d
3. *Brassica oleracea* cv. *capitata* f. *alba*	yellow orange	20/c
6. *Brassica oleracea* v. *gemmifera*	orange	24/d
7. *Brassica pekinensis*	yellow —	8/d
4. *Brassica oleracea* v. *sabauda*	orange	16/d
5. *Brassica oleracea* v. *gongyloides*	yellow orange	20/d
8. *Brassica rapa* ssp. *rapifera*	yellow —	12/d
9. *Brassica napus* f. *annua*	yellow —	14/d
10. *Brassica napus* f. *biennis*	yellow —	10/d
11. *Brassica nigra*	yellow —	4/d
12. *Brassica juncea*	yellow —	4/d
13. *Brassica rapa* f. *campestris*	yellow —	10/d
14. *Brassica carinata*	yellow —	10/c

Seeds of the first four species dissolved in shades of red. The seed solutions of *B. oleracea* v. *sabauda* and v. *gongyloides* were orange coloured.

The colour of solutions obtained from the seeds of *B. napus* f. *annua* and f. *biennis* was — while lighter — similar to that in the former two species. The colour of seed extracts from *B. rapa* f. *campestris* and *B. carinata* was also similar to theirs. On the other hand, seed extracts of *B. nigra*, *B. juncea*, *B. pekinensis* and *B. rapa* ssp. *rapifera* gave light yellow shades of colour.

Since the colour substances of seeds in the different *Brassica* species and varieties gave solutions of different colours in the 1.5 gram molecule solution of NaOH, the colour of the solutions could be used — through a comparison with a colour chart — for the identification of species and varieties from the seed.

The mustard species (*B. nigra*, *B. juncea*) as well as *B. rapa* ssp. *rapifera* and *B. pekinensis* can be easily distinguished from all varieties of *B. oleracea*. From the latter *B. oleracea* v. *sabauda* and *B. oleracea* v. *gongyloides* can also be distinguished. The colour of seed extracts from *B. napus* f. *annua* and f. *biennis* as well as from *B. rapa* f. *campestris* is a similar — though somewhat lighter — orange as that in the two latter varieties of *B. oleracea*. We were rather uncertain in distinguishing the varieties of *B. oleracea:* cv. *acephala*, v. *capitata* f. *rubra* and f. *alba*, v. *gemmifera* of which the seed extracts showed red shades of colour, although there were slight differences in the shades.

3. *Photometric measuring of light absorption by the solutions.* According to the descent of the light absorption curves the species examined can be divided into three groups similarly to the distribution by colour (red, orange, yellow).

a) Varieties producing red solutions: *B. oleracea* convar. *acephala*, v. *capitata* f. *alba* and f. *rubra*, v. *gemmifera*, as well as *B. oleracea* v. *sabauda* which gives an orange solution have similar curves. The characteristic phases of light transmission fall between the wave-lengths of 740—680 and 560—480.

Between the two mentioned wave-length intervals there develops a relatively straight section. There is hardly any difference between the varieties in the light transmission values of the straight sections. They were more different in the shade of their colour.

On the curves of *B. rapa* ssp. *rapifera* and *B. pekinensis* two straight sections develop similarly to the curves of the *B. oleracea* varieties. Their light transmission values are higher in both sections than those of the other *oleracea* varieties.

The case of *B. oleracea* var. *gongyloides* is exceptional. The curve of this variety is straight between the wave-lengths of 740 and 680, but between those of 560 and 480 light transmission gradually decreases. In both wave bands mentioned the values of light transmission are higher than in the case

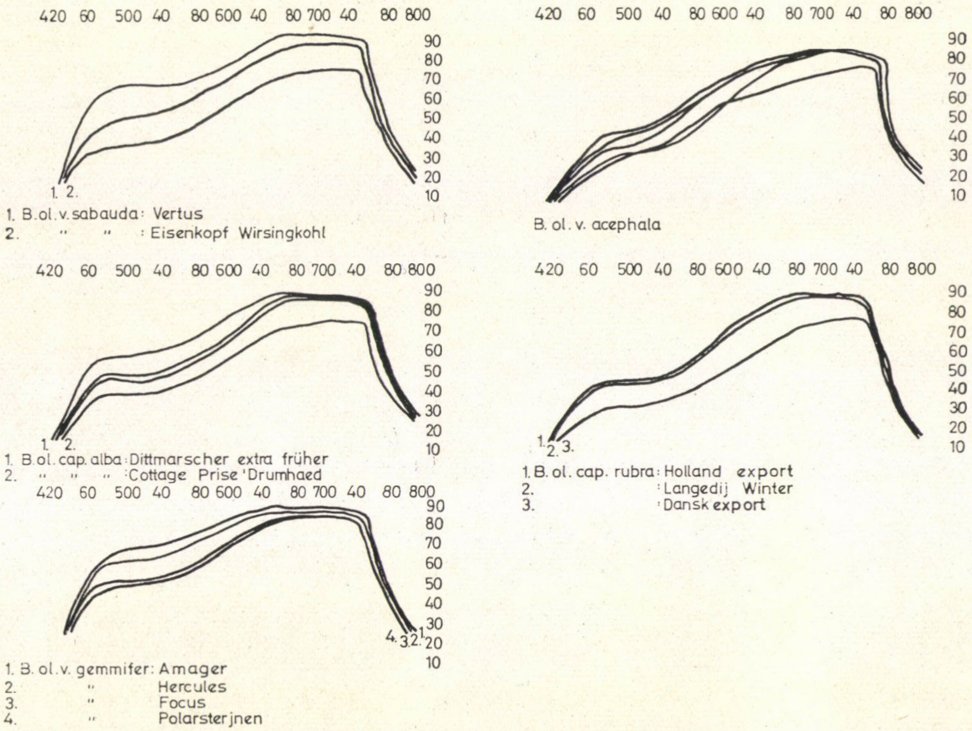


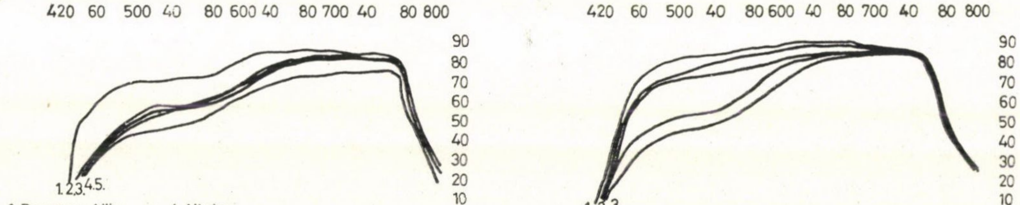
Fig. 2. **Light absorption curves of colour substances in 15 seeds placed in 1.5 gram molecule NaOH, as grouped by the type of curve (varieties of *Brassica oleracea*)**

of the other varieties of *B. oleracea*. Thus, on the basis of the photometric curve this variety is different from the rest; its curve is more like the curves of *B. rapa* ssp. *rapifera* and *B. pekinensis*.

b) On the spectral curves of the two forms of *B. napus* it is only between the wave-lengths of 740-640 micron that a relatively straight light transmission section develops, with the other values light transmission gradually decreases. Thus the curve is similar to that of *B. oleracea* v. *gongyloides*.

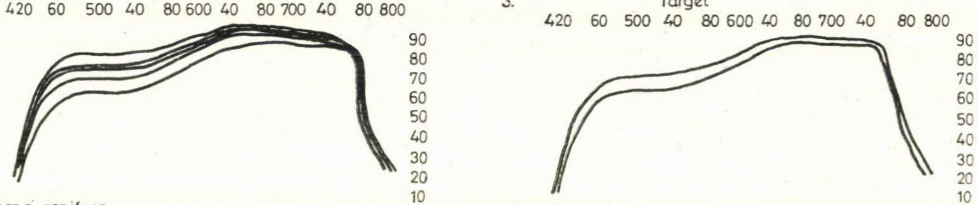
c) The spectral curves of *B. nigra* and *B. juncea* show a light transmission of 96-88 percent between the wave-lengths of 740 and 500 millimicrons, then suddenly fall. As to their curves and colours of solution these two species are highly similar to *B. rapa* ssp. *rapifera*.

The *B. rapa* f. *campestris* has a singular absorption curve. There is a straight line between the wave-lengths of 740 and 680 millimicrons, with a 68—82 percent light transmission value which later gradually decreases (Figs 2, 3, 4).



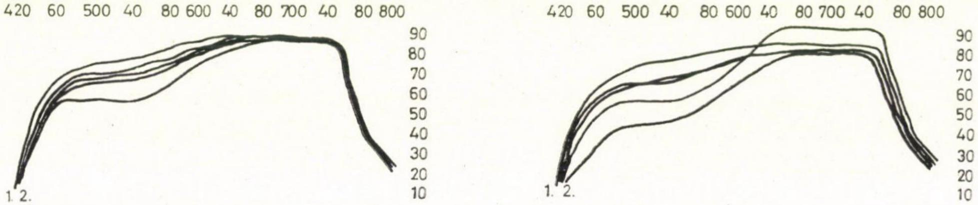
1. B. napus: Allie 4. Victor
2. f. biennis c.l.:v 5. Panter
3. Vestal

1. B. napus f. annue: Gyllen
2. Regina
3. Target



B. rapa rapifera

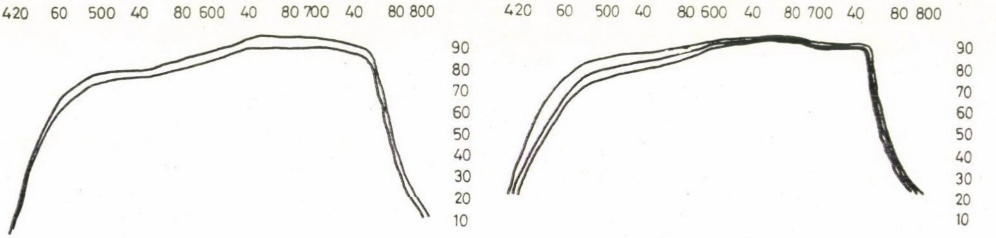
B. pekinensis



1. B. ol. v. caulorapa: Wiener Weisser Treib
2. " : Knudekohl

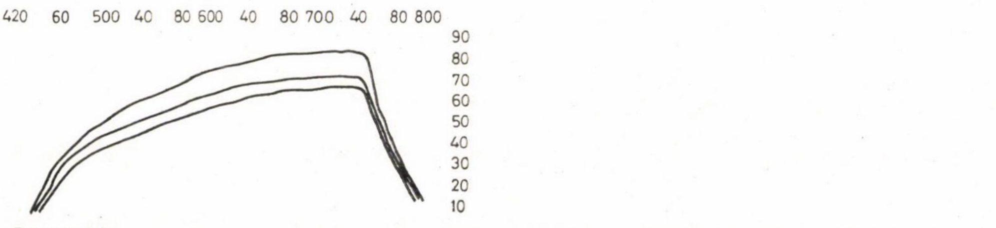
1. B. ol. v. caulorapa: Wiener blauer Treib
2. B. " " —

Fig. 3. **Light absorption curves of colour substances in 15 seeds placed in 1.5 gram molecule NaOH, as grouped by the type of curve (*B. napus* f. *annua*, f. *biennis*, *B. rapa* ssp. *rapifera*, *B. pekinensis*, *B. oleracea* v. *gongyloides*)**



B nigra

B juncea



B campestris

Fig. 4. **Light absorption curves of colour substances in 15 seeds placed in 1.5 gram molecule NaOH, as grouped by the type of curve (*B. nigra*, *B. juncea*, *B. rapa* ssp. *campestris*)**

Conclusions

The colour substances of *Brassica* species and varieties dissolve in NaOH, and the colours of the solutions are markedly or slightly different. The absorbed solution fluoresces in blue or yellow colour.

In daylight the colours of the solutions are so different that — after being compared to a colour chart — they can be used for the identification of species and varieties. Between the red seed extracts of the first four *B. oleracea* varieties there are but slight differences in the shade of the colour. The solutions of *B. oleracea* v. *sabauda* and v. *gongyloides* are of orange shade; those of *B. rapa* f. *campestris* and the two forms of *B. napus* have the same colour. The light yellow solutions of the mustard species: *B. nigra*, *B. juncea*, as well as those of *B. pekinensis*, *B. rapa* ssp. *rapifera* are easily distinguished from the former ones.

VAUGHAN—DENFORD (1968) on the basis of their study on the proteins of the *Brassica* genus, and SIKKA (1940) in the course of genetic researches pointed out that *B. napus*, *B. juncea* and *B. carinata* are amphidiploids and the parents are: *B. rapa* f. *campestris*, *B. nigra* and *B. oleracea*. Solutions made of the colour substances of seeds in the amphidiploids either resemble the seed extract of one of the parents, or show an intermediate colour. E.g.:

B. rapa f. *campestris* × *B. oleracea* = *B. napus*
orange red = orange solution
B. nigra × *B. rapa* f. *campestris* = *B. juncea*
light yellow orange = light yellow solution

In the former two species it can be seen that the spectral curve of *B. napus* is a transition between the curve types of *B. rapa* f. *campestris* and *B. oleracea*.

Studies on the solutions made of the colour substances of seeds in amphidiploids and their parents are worth being continued.

Investigations concerning a number of *Brassica* species and varieties are going on.

Acknowledgement

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CLIMATIC MODEL FOR PHYTOTRON STUDIES

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The author presents the main characteristics of the phytotron under construction at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár. For the models of the climatic factors controlled in the phytotron the climatic series of the Agrometeorological Observatory, Martonvásár and the National Institute of Meteorology, Budapest were used. The author's method: the adaptation of trigonometric functions is described in detail. Calculations were carried out with a computer. Of the results the annual course of observations at 7 a.m. and 1 p.m. representing the extreme values, while of the daily courses January and June representing — annually — similarly extreme values are presented. The accuracy of the approach corresponds to the adjustment of elements programmable in the phytotron.

Introduction

The effects of phenomena of weather and climate on the growth and development of plants under field conditions can be studied only in a descriptive way. The conditions of the experiment, the elements of the weather are eventualities impossible to determine in advance by the researcher. The experiments cannot be repeated under the same natural conditions. Agro- and biometeorological researches will become exact only when carried out in modern air-conditioned phytotrons where of the elements of weather the most important ones: air temperature, light and air humidity can be controlled at will. In such a system the effect of any component can be studied with the others kept simultaneously at a constant level. In this way the experiments can be repeated and purposefully laid out. The system of plant growing chambers, cases, air-conditioned glass-houses and artificially illuminated open spaces made for a definite purpose of research is called a phytotron.

The first equipment that can be called a real phytotron has been operating since June 1949 in Pasadena (USA, California). Since then many phytotrons have been constructed all over the world in which the effect of weather conditions on plants can be studied in an exact way. The various types of phytotron are described by Nitsch (1969).

The equipment was ordered and the construction started after many-sided scientific investigations including the methodological research initiated in 1968 for the preparation of meteorological programmes (O'SVÁTH—PLETSER 1970).

The phytotron constructed at Martonvásár is a two-storied building of 50×50 m ground space in which an unsupported hall of 30×30 m — i.e. 900 m^2 — area is placed at the centre of the upper floor, with the air conditioned units. The 44 air conditioned units placed in the central hall are supplied with air, water and electric power from the specially equipped rooms of a space (of the same size as the hall) located under the hall in the middle of the ground floor. On two floors around the central hall there are laboratories and researchers' rooms where plants are prepared for the experiments and those removed from the air-conditioned units processed or further analysed.

In the central hall the air-conditioned glass-houses are replaced by plant growing chambers where — with the uncontrollable sunshine excluded — light intensity can be programmed in three steps by artificial illumination of a maximum of 50,000 lux. Air temperature can be controlled between 15 and 35°C. The air-conditioning equipment is built into the plant growing benches. Plant growing benches placed in the 14 rooms have a total useful surface of 60.2 m^2.

The heart of the phytotron: 12 air-conditioned plant growing chambers and 16 similar cabinets are placed again in the central hall. Their total useful plant growing surface is 62 m^2 of which the chambers have 39.6 m^2, the cabinets 22.4 m^2. The chambers are of "spring-autumn" type, with a temperature adjustable from -5°C to $+40$°C. The cabinets are of "summer" type, with an air temperature controlled between 5° and 40°C. The maximum light intensity attainable in the chambers and cabinets is generally 50,000 lux, in 4 cabinets 100,000 lux which corresponds to maximum light intensity in summer. In these too, the light intensity can be programmed in three steps, but by hand switching can be set at 15 stages.

The air-conditioned plant growing units are completed by 2 chambers for studies on frost resistance. Their total useful surface is 14.2 m^2. Minimum temperature attainable in these chambers is -25°C.

With the exception of the above mentioned "winter" chambers air humidity can also be programmed in all the 42 plant growing units.

In the air-conditioned units maximum deviation from the programmed value at the given level may be ± 0.5°C in the case of temperature, ± 5 per cent with light intensity and ± 3 per cent with air humidity. Each unit has a separate built-in air-conditioning equipment which corrects deviations from the programmed temperature and air humidity automatically. Possible deviation from the programmed light intensity can be corrected with the vertical shifting of the lighting ceiling, by means of a photometer.

When planning the experiments to be performed — starting from the original conception — the aim is to get an exact, scientific knowledge of metabolism and hereditariness, more closely, to answer these cardinal questions of genetics and evolution. This subject includes some important agronomic questions: autumnization-vernalization, frost resistance and winter-hardiness, vegetative period, quality, resistance, etc., their scientific foundation and the use of methods, thus developed, in practical plant breeding (Rajki 1966, 1967; Rajki—Dévay—Rajki 1972).

Material and Method

The task is to prepare an average meteorological model serving — as the basis of the phytotron work — for the comparison of the special programmes. The average meteorological data can be obtained from a long series of data. On the other hand, efforts should be made to give as detailed information as possible. With meteorological data this requirement can be met by the utilization of hourly averages. At Martonvásár, the site of the phytotron, series of meteorological data satisfying all requirements are not yet available. The nearest place long meteorological series can be obtained from is Budapest. The monthly per hour averages of air temperature and humidity are taken from the 1931—1950 year-books of the National Meteorological Institute. Total hours per month of sunshine and sky radiation can also be found in the year-books. The publication of these data began in 1936. Average number of hours per month in the period between 1936 and 1960 is contained in "Magyarország éghajlati atlasza II. k. Adattár" (Meteorological Map of Hungary. Vol. II. Collection of data), expressed in gcal/cm^2 units. Our meteorological model was thus prepared using a meteorological data series of 20 years for the hourly values of air temperature and humidity per month and one of 25 years for the radiation. As a meteorological series these series cannot be considered very long, but when evaluated on the basis of the literature of the phytotron they seem sufficient. Bretschneider—Hermann (1969) prepared e.g. the climatic programme of the Rauisch-Holzhausen Phytotron from 15 years' meteorological data. From the data series of climatic observations made at Martonvásár meteorological data obtained since 1955 are available. Of them the annual course of daily means of air temperature and that of the daily means of soil temperature measured in the 5 cm soil layer have been processed.

Daily and annual courses of weather components can easily be approached by the adaptation of trigonometric functions. Periodicity is caused by the rotation of the earth and its revolution round the sun. Periodicity can be demonstrated even for non-continuous weather components as proved by Jordan (1949) with trigonometric functions adapted to the precipitation series of 1871—1940, Budapest.

Takács (1967) adapted trigonometric functions to the daily course of soil temperature. Bliss (1958) studied the regression of biological and climatological periods by applying trigonometric functions.

O'sváth—Pletser (1970) determined the annual course of monthly means of soil temperature measured in the upper 5 cm layer at the climatic station of the Agrometeorological Observatory at Martonvásár from a 13 years series of data, by applying trigonometric functions.

The theoretical bases of the method are described by many hand-books of which two are mentioned here: those by Bermant (1951) and Jordan (1956) which served as sources for the present paper.

Suppose that x phases — in the present case concrete periods: hours, days, pentades, decades or months — and the pertaining $y(x)$ quantities — here the hourly value, daily mean, etc. of the weather component — are given. The x phases are numbered $x = 0, 1, 2 \ldots$ up to $N - 1$. This practically means that when studying the daily course we mark the 1st hour with 0, the 2nd with 1 . . . the 24th with 23. In this case N is equal to the number of phases i.e. 24. The same applies to other units of time: days, pentades and months too; y should be approached to by the following $f(x)$ function:

$$f(x) + a_0 \varphi_1(x) + \varphi_2(x) + \ldots\ldots + \varphi_n(x),$$

where

$$\varphi_m(x) = a_m \cos \frac{2\pi\, mx}{N} + b_m \sin \frac{2\pi\, mx}{N}\,.$$

This is the mth wave the wave-length of which is N/m, namely $\varphi_m(x) = \left(\varphi_m x + \frac{N}{m}\right)$.

Suppose that $2\pi mx/N = \omega$ and let us determine the extreme values of φ_m. In this case $D\varphi_m = -a_m \sin\omega + b_m \cos\omega = 0$, that is $\operatorname{tg}\omega = b_m/a_m$. If the quantity corresponding to one of the extreme values of the mth wave is marked with ω_m, then $\omega_m + k$ is also an extreme value.

The φ_m wave can be expressed in another way too:

$$\varphi_m = a_m^2 + b_m^2 \cos(\omega - \omega_m),$$

where $\sqrt{a_m^2 + b_m^2} = r_m$ is the amplitude of the wave, that is half of the difference between its highest and lowest points. Of this $\varphi_m = r_m \cos(\omega - \omega_m)$, where $\omega = 2m\pi x/N$.

The wave is thus given by the wave-length, the amplitude and the place of the maximum. If the amplitude is equal to one, we obtain the sinus wave; if it is larger than one the wave is strong, if smaller than a half, then it is weak.

Approximation is carried out in accordance with the principle of the least square, that is by reducing the sum of the deviation squares to the minimum:

$$S = \sum_{x=0}^{N} [f(x) - y]^2.$$

Factors a_m and b_m giving the extreme values are calculated by means of normal equations:

$$\frac{\partial S}{\partial a_m} = 0 \quad \text{and} \quad \frac{\partial S}{\partial b_y} = 0$$

$m = 0, 1, 2, \ldots n$ and $y = 1, 2, \ldots n$, which gives a $2n + 1$ equation, determining the unknown $2n + 1$. The extreme value will be a minimum if

$$\sum \sum \frac{\partial^2 S}{\partial a_m \partial b} da_m\, db_y > 0,$$

where the sum includes all double combinations of the quantities a_m and b_y.

Normal equations can be cancelled through the orthogonality of the functions (BERMANT 1951, JORDAN 1956). After all the extent of approximation can be calculated from the following formula:

$$\sigma^2 = \sum_{x=0}^{N} \frac{y^2}{N} - a_0^2 - \frac{1}{2} \sum_{m=1}^{n+1} (a_m^2 + b_m^2).$$

The approximative function is

$$\varphi_i(x) = a_0 + \sum_{i=1}^{n} \left(a_i \cos\frac{i\,2\pi\,x}{N} + b_i \sin\frac{i\,2\pi\,x}{N}\right),$$

where a_0 is the average of the y values measured, i the number of waves used for the approximation, N the number of periods, while a_i and b_i can be calculated from the following formulas:

$$ai = \frac{2}{N} \sum_{x=0}^{N-1} y \cdot \cos\frac{i\,2\pi\,x}{N}, \qquad b_i = \frac{2}{N} \sum_{x=0}^{N-1} y \cdot \sin\frac{i\,2\pi\,x}{N},$$

For the sake of a simpler way of writing let us mark the cosinus members with u_i, and the sinus members with v_i.

Before the computation let us prepare the table of u and v values. The u and v values pertaining to the equal intervals of $N = 24$, 12, 8, 7 and 6 are shown in Table 1. For the calculation of factors a_i and b_i the y values had better be arranged in groups, since the u and v constants are periodically repeated. With the increase of i the a_i and b_i factors approach to 0. In practice, however, no i = infinity wave has to be used for our approximative function. The extent of approximation is generally satisfactory with three waves.

In the case of $N = 24$ (daily course of hourly values) and $i = 3$ wave the factors can be calculated with simple arithmetical operations:

$$a_0 = \frac{y_0 + y_1 + y_2 + \ldots y_{23}}{24}$$

Table 1

u and v values in equal intervals of N = 24, 12, 8, 7 and 6 from x = 0 to x = N—1

x	N = 24		N = 12		N = 8		N = 7		N = 6	
	u	v	u	v	u	v	u	v	u	v
0	1	0	1	0	1	0	1	0	1	0
1	0.966	0.259	0.866	0.5	0.707	0.707	0.624	0.782	0.5	0.866
2	0.866	0.5	0.5	0.866	0	1	—0.225	0.975	0.5	0.866
3	0.707	0.707	0	1	—0.707	0.707	—0.901	0.434	—1	0
4	0.5	0.866	—0.5	0.866	—1	0	—0.901	—0.434	—0.5	—0.866
5	0.259	0.966	—0.866	0.5	—0.707	—0.707	—0.225	—0.975	0.5	—0.866
6	0	1	—1	0	0	—1	0.624	—0.782		
7	—0.259	0.966	—0.866	—0.5	0.707	—0.707				
8	—0.5	0.866	—0.5	—0.866						
9	—0.707	0.707	0	—1						
10	—0.866	0.5	0.5	—0.866						
11	—0.966	0.259	0.866	—0.5						
12	—1	0								
13	—0.966	—0.259								
14	—0.866	—0.5								
15	—0.707	—0.707								
16	—0.5	—0.866								
17	—0.259	—0.966								
18	0	—1								
19	0.259	—0.966								
20	0.5	—0.866								
21	0.707	—0.707								
22	0.866	—0.5								
23	0.966	—0.259								

$$a_1 = 1/12\,[(y_0 - y_{12}) + 0.966\,(y_1 - y_{11} - y_{13} + y_{23}) + 0.866\,(y_2 - y_{10} - y_{14} + y_{22}) +$$

$$+ 0.707\,(y_3 - y_9 - y_{15} + y_{21}) + 0.5\,(y_4 - y_8 - y_{16} + y_{20}) +$$

$$+ 0.259\,(y_5 - y_7 - y_{17} + y_{19})]$$

$$b_1 = 1/12\,[(y_6 - y_{18}) + 0.966\,(y_6 + y_7 - y_{17} - y_{19}) + 0.866\,(y_4 + y_8 - y_{16} - y_{20}) +$$

$$+ 0.707(y_3 + y_9 - y_{15} - y_{21}) + 0.5(y_2 + y_{10} - y_{14} - y_{22}) +$$

$$+ 0.259(y_1 + y_{11} - y_{13} - y_{23})]$$

$$a_2 = 1/12[(y_0 - y_6 + y_{12} - y_{18}) + 0.866(y_1 - y_5 + y_7 + y_{11} - y_{13} - y_{17} + y_{19} + y_{23}) +$$

$$+ 0.5(y_2 - y_4 + y_8 + y_{10} - y_{14} - y_{16} + y_{20} + y_{22})]$$

$$b_2 = 1/12[(y_3 - y_9 + y_{15} - y_{21}) + 0.866(y_1 + y_5 - y_7 - y_{11} + y_{13} + y_{17} - y_{19} - y_{23}) + \\ + 0.5(y_2 + y_4 - y_8 - y_{10} + y_{14} + y_{16} - y_{20} - y_{22})]$$

$$a_3 = 1/12[(y_0 - y_3 + y_6 - y_9 + y_{12} - y_{15} + y_{18} - y_{21}) + \\ + 0.5(y_1 - y_2 - y_4 + y_5 + y_7 - y_8 - y_{10} + y_{11} + y_{13} - y_{14} - y_{16} + y_{17} + y_{19} - y_{20} - \\ - y_{22} + y_{23})]$$

$$b_3 = 0.866/12(y_1 + y_2 - y_4 - y_5 + y_7 + y_8 - y_{10} - y_{11} + y_{13} + y_{14} - y_{16} - y_{17} + \\ + y_{19} + y_{20} - y_{22} - y_{23})$$

The three waves correspond in this case to a one day, half a day and quarter of a day period respectively. The calculation requires much work even in the above simplified form, so it had better be performed with a computer. After the coefficients had been computed, the series of numbers of meteorological models were obtained again by means of a computer, on the basis of the approximative function already presented.

A special problem was represented by the modelling of the radiation data. On the basis of the data available the daily course of radiation can be regarded as continuous only from sunrise to sunset. If there were as long a series of data available on eradiation as on irradiation, then the balance of radiation would be continuous. As it is, however, we had to find a solution by which the course of radiation could be modelled. The beginning and end of radiation was determined from the monthly average number of hours of sun and sky radiation by the application of trigonometric functions. For this purpose each row of monthly average number of hours was completed with a zero at both ends. In accordance with the facts we considered irradiation before sunrise and after sunset as zero. Negative figures obtained from the approximate values were not taken into account in the process of modelling, as irradiation can be nothing else but positive.

When preparing our model we set the "change of weather" to occur every five days. A five-day change means a fairly small step and approaches the natural conditions rather well, since weather conditions in Hungary have a tendency to last for five days. Programme modification carried out every five days is suitable for the operation of the phytotron too.

In the equations of the annual courses of average numbers of hours per month the five-day intervals were displaced by the $N - 73$ period. The respective cosinus and sinus values were programmed and applied — by means of a computer — to the equations of the appropriate functions. Thus, after all, the model surfaces limited originally by trigonometric function curves will be formed after the transformation by planes. The position in space of these planes can be imagined and illustrated as that of the upper surface of a rectangular brick-shaped prism. The height of the prism is determined by the value of the weather component studied, its width by the number of hours — in the present case one hour — and its length by the number of days, — five days according to the value chosen.

Results

It is seen from what have been told that during the work of processing such a large amount of numerical data was obtained as cannot be fully presented. Application of the equations will be shown through some examples. We began to deal with the methodological part of the work of modelling as early as in 1968. During this work it was to the decade means of air temperature measured at the Agrometeorological Observatory of Martonvásár from 1955 to 1967 that a trigonometric function was applied, with the following equation:

$$Y_i = 10.5084 - 10.9574 \cdot u_1 - 2.3176 \cdot v_1.$$

Fig. 1 shows the application of the measured and computed values. The difference is the largest at the maximum and minimum. This may be caused by the fact that 13 years data are insufficient to eliminate the effect of some years with extreme weather conditions. Adjustment, especially around the maximum and minimum, could be improved by one or two more waves added. Today a series longer by four years is already available, but changing the time of observation from the earlier 7, 14 and 21 hours to 7, 13 and 19 hours has made the series heterogeneous. The function can be used for the simplest temperature programmes.

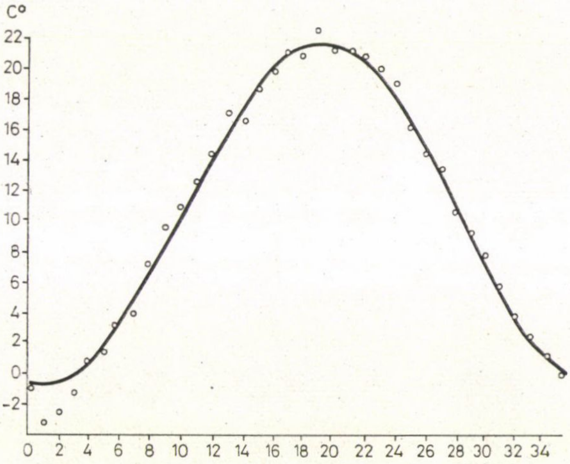

Fig. 1. Measured (○) and computed (—) values of the decade means of air temperatures. Martonvásár 1955—1967

Values — measured and computed with trigonometric functions applied — of monthly means of soil temperatures measured 5 cm deep at the climatic station of the Agrometeorological Observatory of Martonvásár from 1955 to 1967 are presented from our earlier paper (O'SVÁTH—PLETSER 1970) on Fig. 2. The measured values agree with the curve constructed on the basis of values computed from the function $Y = 10.6237 - 11.3063 \cdot u_1 - 0.8535 \cdot v_1$. Considerable differences cannot be found even around the maxima and minima. The trend of soil temperature can thus be approximated with a single wave.

Fig. 3 shows the monthly averages of hourly amounts of total radiation in January and June, and the three-wave curves adjusted to them. The equations are:

$$Y_{January} = 172.25 - 209.83u_1 + 4.77v_1 + 15.33u_2 + 3.75v_2 + 12.67u_3 - 2.50v_3,$$

$$Y_{June} = 924.00 - 756.50u_1 + 172.96v_1 - 104.68u_2 + 16.32v_2 - 8.75u_3 - 0.25v_3.$$

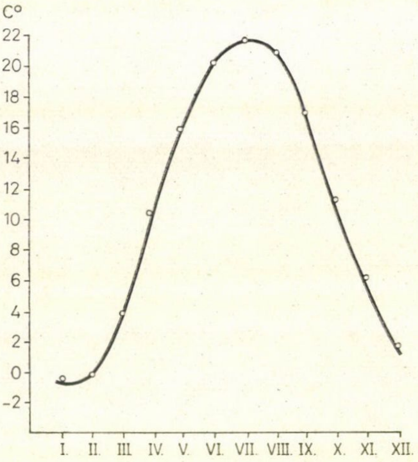

Fig. 2. Measured (○) and computed (—) values of monthly means of soil temperature in the upper 5 cm soil layer. Martonvásár 1955—1967

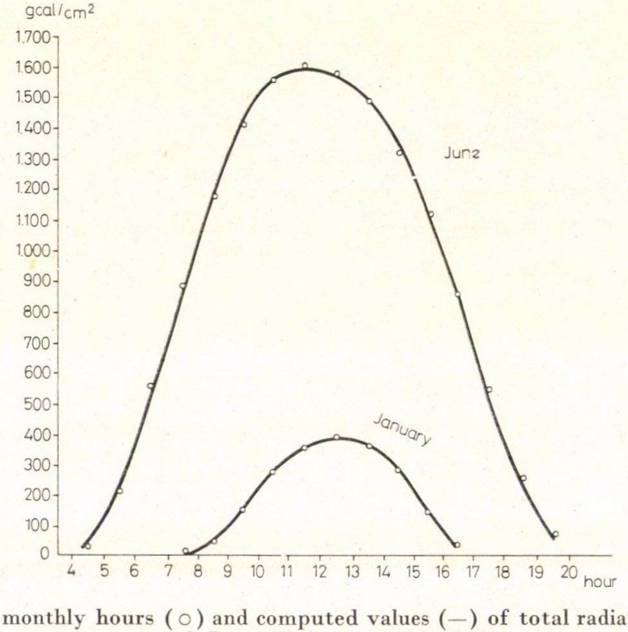

Fig. 3. Average monthly hours (○) and computed values (—) of total radiation in January and June. Budapest 1936—1960

When computing $Y_{January}$ we assumed periods of 12, 6 and 3 hours. The first wave has the highest a_1 factor, those of the second and third waves are smaller by several orders of magnitude; thus periods of half and quarter of a day, respectively, affect the daily trend of radiation only to a low extent in January.

When computing Y_{June} we assumed periods of 16, 8 and 4 hours. This curve is not symmetric either. In the daily trend of January the descending branch, while in June the ascending branch is more steep. In the latter the factors of the second wave are rather high too, but those of the third are lower by several orders of magnitude.

Approximation of the daily trend of radiation has been presented for a winter and a summer month. The approximation can be considered satisfactory as it attains the exactness of instrument reading. Any point of the curves drawn can be computed with the given equations, so errors originating from a subjective graphic connection of the points can be eliminated. The equations are suitable for computing the linear regression with them. From our measurements of light intensity — having collected a sufficient number of data — we can calculate correlations with the daily and annual trends of light intensity and irradiation. And with the regression equation in possession we can calculate the average daily and annual changes of light intensity.

When preparing the model of air temperature we started from 20 years monthly averages per hour of the Budapest series of meteorological data. To the annual trend of each hourly value of the monthly averages a two-wave trigonometric function was adjusted, then in the resulting 24 equations the cosinuses and sinuses of angular values corresponding to the five-day intervals were substituted. In that way the hourly means were obtained for all pentades and all 24 hours, respectively, of the year.

Of the annual trend of hourly means of pentades the yearly course of the values of 7 a.m. and 1 p.m. is presented by Fig. 4. The equations are:

$$Y_7\ \text{a.m.} = 8.455 - 10.627u_1 - 0.184v_1 - 0.142u_2 - 0.323v_2,$$

$$Y_1\ \text{p.m.} = 14.378 - 13.229u_1 + 0.130v_1 - 2.253u_2 - 2.881v_2.$$

Adjustment of the curves to the starting point — the 20 years average of monthly means per hour — can be regarded as satisfactory, since it does not exceed the accuracy of thermographic measurement: ±0.5°C. To describe the annual trends of pentade means of temperatures measured at 7 a.m. two waves seem to be sufficient, as the coefficients of the second wave are relatively low. This does not apply to the curve of 1 p.m. since its coefficients are significant even in the second wave. Application of a third wave would probably improve the approach. The computer programme for a three-wave approximation has already been prepared, and the calculations will be completed soon.

The model of the daily and annual trends of air humidity has been made from the same series of meteorological data and with the same method as that of the air temperature. The annual trend of pentade means of relative

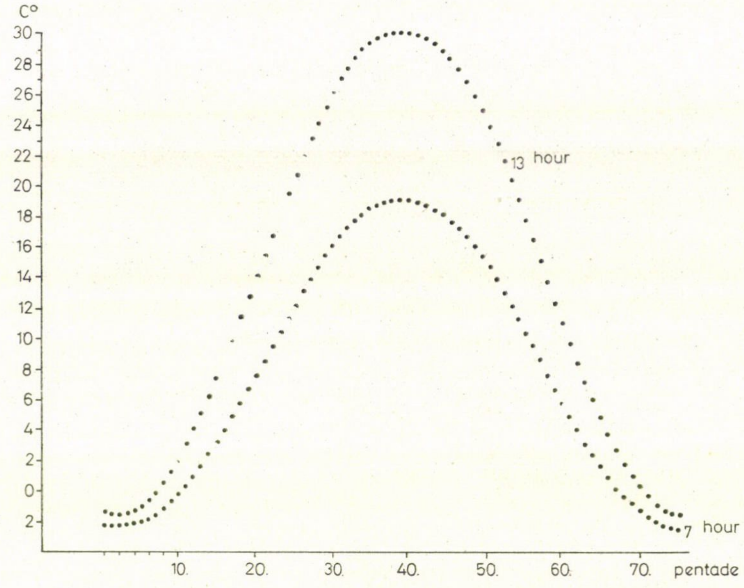

Fig. 4. Computed pentade means of air temperature at 7 a.m. and 1 p.m.

air humidity measured at 7 a.m. and 1 p.m. is shown by Fig. 5. The equations are:

$$Y_7 \text{ a.m.} = 78.375 + 6.633u_1 - 5.414v_1 - 0.338u_2 + 1.544v_2,$$

$$Y_1 \text{ p.m.} = 58.250 + 15.743u_1 - 5.691v_1 + 4.150u_2 - 0.346v_2.$$

Adjustment is ±2 per cent. From the coefficients of the equations the conclusion can be drawn again that an additional wave would improve the approximation.

A three-wave trigonometric function applied to the annual trend corresponds to periods of one year, half a year and a quarter of a year. The first wave is justified by the one year periodicity of a day's length. The good adaptation to the half-year period is explained by the vernal and autumnal equinox dividing the year into two parts. The actual physical cause of the third — quarterly — wave is no longer so unequivocal; we even may attribute the improvement of adaptation to mathematical rather than physical causes. When approximating the daily trend we assumed periods of 24, 12 and 6 hours. Of them only the first can be justified physically, and even that only for the equinoctial days. Waves of the daily trends of weather components considerably deviate in every other case from the regular sinus curve. The three-wave function adapted to the monthly means of hours of many years' averages gives a good approximation in spite of what have been told.

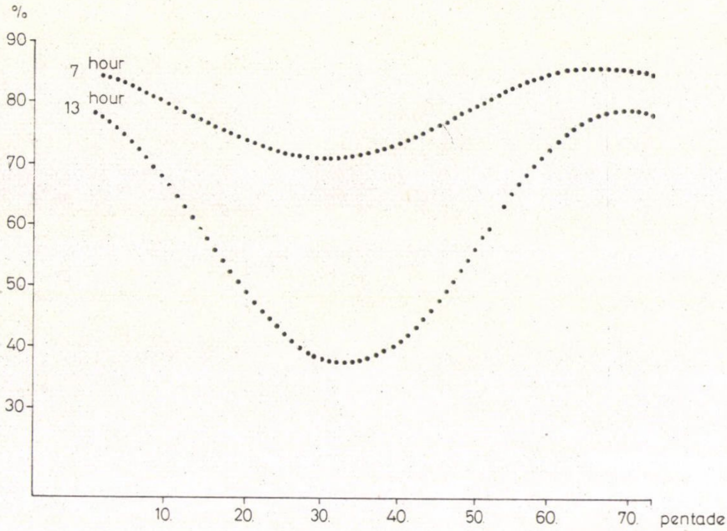

Fig. 5. Computed pentade means of relative air humidity at 7 a.m. and 1 p.m.

Of the three-wave trigonometric functions adapted to the twenty years' average of monthly mean air temperatures per hour those prepared by the use of January and June data representing the extreme values are shown in Fig. 6. The unbroken line indicates the calculated, the circles the experienced values. The equations are:

$$Y_{January} = 1.74 - 0.83u_1 - 1.04v_1 + 0.35u_2 + 0.05v_2 - 0.08u_3 - 0.10v_3,$$

$$Y_{June} = 19.78 - 3.43u_1 - 1.64v_1 + 0.43u_2 - 0.12v_2 - 0.03u_3 + 1.08v_3.$$

The adaptation is better than with the two-wave approximation, deviation reaches ±0.2°C only in a few cases. In the large amplitude June wave the coefficients of the third period are no less significant, as the multiplier of v_3, the coefficient b_3 may in some cases increase or decrease by almost one degree the approximate value of the mean temperature pertaining to the given hour. The coefficients of the third wave in the function approximating the January trend are much lower, but here the mean is also low, so the three-wave approximation is justified here too.

The January and June averages of relative air humidity and their approximate values are shown in Fig. 7. The equations are:

$$Y_{January} = 80.87 + 2.90u_1 + 2.04v_1 - 0.40u_2 - 0.48v_2 - 0.05u_3 + 0.78v_3,$$

$$Y_{June} = 62.08 + 11.4u_1 + 5.78v_1 - 0.41u_2 - 0.04v_2 + 0.50u_3 - 0.35v_3.$$

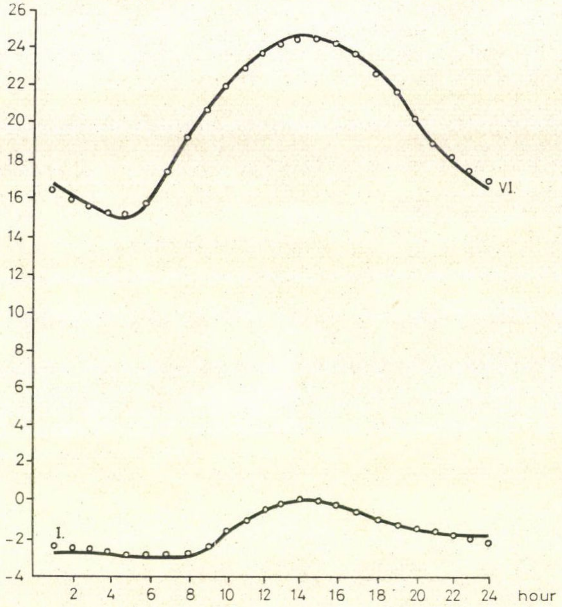

Fig. 6. Monthly averages (○) and computed values (—) of hourly air temperatures in January and June. Budapest 1931—1950

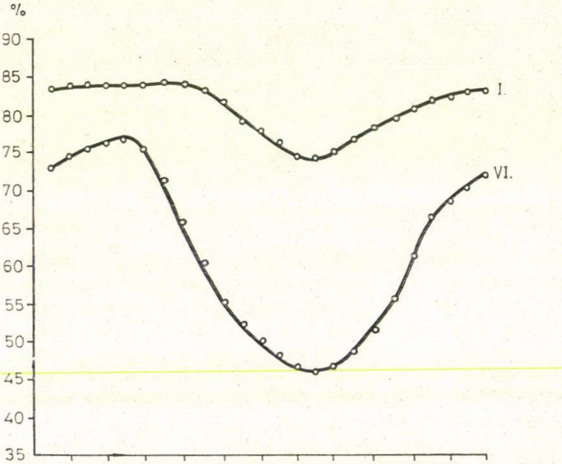

Fig. 7. Monthly averages (○) and computed values (—) of hourly relative humidity in January and June. Budapest 1931—1950

The points of the experienced values marked with circles agree with the approximated values marked with an unbroken line. Deviations reach 1 per cent only around the extreme values. The coefficients of the second and third wave are rather low indicating that in the daily trend of air humidity the 24-hour period is dominant.

Thus, after all, the adaptation of the three-wave functions is the most suitable for the preparation of the model. Further investigations are required to answer the question whether by means of the equations obtained the hourly values of weather components can be sufficiently approximated at places where no hourly measurements only the a_0 values of the equations — i.e. the monthly means — are available.

In this case we have to assume that the equation approximating the hourly trend of the weather component studied differs from that of the initial material only in the average, that is, only the average is modified by the local effects. Of course, climatic differences cannot be expected to show only in the averages, but it would be useful to know the adaptation of such an approximation as well. The calculation data of places where hourly observations have also been made can be used as a control.

Substitution of mean values of extremely cold or warm periods into equations describing the average course is a similar problem. The question is to what extent the hourly means of a cold, warm, wet or dry month can be approximated with a function computed from the average.

The model outlined here is thus the first approach to the meteorological programming of the phytotron. With our further activity we shall make efforts that the mathematically described model should approach the natural conditions as much as possible. This requires — in addition to the processing of climatic data — a lot of measuring work to be carried out both in the field and in the phytotron.

Acknowledgement

We are indebted to Sándor Rajki dr., director, for helping us in our work with his advice, by putting the literature of phytotrons at our disposal and by giving information on the technical and scientific objectives of the Martonvásár Phytotron.

In the processing of data great assistance was given by László Gáspár dr., scientific department leader who prepared the first computer programme and introduced us into the handling and programming of the computer.

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DYNAMICS OF BLOSSOMING AND FERTILITY OF PISTILS IN PEAR VARIETIES

By

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Dynamics of blossoming and fertility of pistils in 12 pear varieties were studied between 1968 and 1970. There were annually varying differences between the varieties in the beginning of flowering, the extent of simultaneous blossoming and the time of full bloom. On the average of the years examined the pear varieties required a total heat of 3510.5°C to begin flowering. Pistils were fertile for two or three days depending on the meteorological factors, "wilting" (browning and drying up) set in rapidly — in one or two days. Dehiscence of anthers within the same flower lasted for 1—2 days in bright weather, and 4—5 days in rainy, cool weather. In the pistils sexual maturity set in 1—4 days earlier than in the anthers.

Introduction

With a view to attain adequate yields it is indispensable to know the dynamics of flowering and the simultaneous blossoming of pollen varieties, especially in the case of totally self-sterile pear varieties.

Time of blossoming, dynamics of flower opening, fertility of pistils and dehiscence of anthers in the different varieties are influenced by climatic factors — first of all by the temperature.

The possibility of successful pollination depends to a great extent on whether the pollen donor and pollen receptor variety are in full blossom at the same time, that is, the duration of pistil fertility in the receptor, and the time of pollen dispersion in the donor variety coincide.

Material and Method

The investigations were carried out at the Érd-Elvira Station of the Horticultural Research Institute between 1968 and 1970 with five trees each pear variety grafted to wild pear seed stocks planted in 1953. The investigations included the following pear varieties: Bosc kobak, Clapp kedveltje, Diel vaj, Dupuit asszony, Favrené asszony, Hardenpont téli vaj, Nemes Krasszán, Pap körte, Pringalle vaj, Serres Olivér, Téli esperes and Vilmos körte.

The opening of the flowers was followed with attention every morning between 8 and 10 a.m. on 1000—1500 flowers selected on the southern side of the crowns at medium height. The number of flowers opened, in full bloom, and withered was recorded. Flowers were considered to be in full bloom when the secretory activity of the pistils had started and the pistils were fertile in them, while flowers in which the pistils had "wilted" (browned and dried up) were regarded as withered. The duration of pistil fertility and the time of dehiscence of anthers were studied with 5000 flowers of the Bosc kobak clone No. 13/1.

Temperatures were registered by a thermograph. Heat amounts required for the beginning of flowering were determined with the method developed by HERBST—WEGER (1940) for pears.

Characterization of the soil. At the Érd-Elvira Station of the Horticultural Research Institute the soil is of meadow origin, slightly alkaline, here and there neutral (pH 6.5—7.5); its $CaCO_3$ content ranges from low to high percentages (5—25%). Its humus content is satisfactory, being 3—4 per cent or occasionally even more in the surface soil. As to its texture the soil is light or medium hard clay of granular structure. In the surface soil the amount of readily available phosphorus (P_2O_5) is 3—8 mg/100 g soil, while that of potassium (K_2O) is 9—16 mg/100 g soil; that is, the soil is moderately, and here and there even poorly supplied with available phosphorus and potassium. The total N content of the soil ranges between 0.30 and 0.40 per cent, P_2O_5 content between 0.12 and 0.17 per cent, K_2O content from 0.90 to 1.20 per cent.

General characterization of the climate. The average temperature of many years on the area in question is 11.6°C. The annual amount of precipitation ranges from 550 to 600 mm which is below the national average, and its distribution is highly uneven. A bright sky is frequent, there are abundant sunshine hours with intensive radiation. The mean values of air humidity are relatively low and promote daily and annual temperature fluctuations. The prevailing wind is of north-west direction and medium strength.

Heat amounts determining the time of blossoming and average values of daily mean temperatures influencing the dynamics of flower opening are given in Table 4.

Results

Time of blossoming and dynamics of flower opening in the varrous pear varieties. The time of blossoming is genetically determined in the fruit species and varieties. Its calendar date is mainly determined by the temperature trend — the amount of heat required for the different biological stages (BRÓZIK—NYÉKI 1970). The beginning of flowering can be modified by the number of sunshine hours, the amount of precipitation, the soil temperature, heat loss due to radiation at night, the dormant state of the varieties, the rate of rise in temperature, etc. (HEGYFOKI 1926, SCHNELLE 1955, TAMÁS 1959, BUDIG 1960, MITTEMPERGHER *et al.* 1965, GRIGGS—IWAKIRI 1969, BRÓZIK—NYÉKI 1970).

Flowering times observed in 250—410 pear varieties were analysed in detail between 1949 and 1969 (BRÓZIK—NYÉKI 1970). Differences of 3—20 days (!) found between the varieties in the time of full bloom made a thorough study of the dynamics of flower opening necessary.

The results are presented in Tables 1, 2 and 3. They show annually changing differences between the varieties in the beginning of flowering and the extent of simultaneous blossoming.

Though the relative order of the varieties is rather stable in accordance with BROWN's data (1943), certain varieties (e.g. Diel vaj, Favrené asszony, Pap körte, Téli esperes) may display annual changes in the time of blossoming, as pointed out by HERBST—WEGER (1940).

Flowering forecast on the basis of heat amounts. There are various

Table 1

Flower opening dynamics of pear varieties as expressed by the percentage of flowers opening on subsequent days (1968)

Érd-Elvira

Variety	April								
	14	15	16	17	18	19	20	21	22
1. Bosc kobak		0	8	10	15	65	97		100
2. Clapp kedveltje		0	5	8	10	60	95		100
3. Diel vaj	0	1	15	45	60	80			100
4. Dupuit asszony			0	5	15	50	70		100
5. Favrené asszony		0	5	37	50	76			100
6. Hardenpont téli vaj		0	8	19	30	85		100	
7. Nemes Krasszán		0	8	40	65	93			100
8. Pap körte		0	10	45	70	83			100
9. Pringalle vaj		0	2	10	15	63	90		100
10. Serres Olivér		0	5	62	85	95			100
11. Téli esperes	0	2	15	60	85	97			100
12. Vilmos körte			0	10	18	70	99	100	

Table 2

Flower opening dynamics of pear varieties as expressed in the percentage proportion of flowers opening on subsequent days (1969)

Érd-Elvira

Variety	April				May		
	27	28	29	30	1	2	3
1. Bosc kobak			0	10	85	100	
2. Clapp kedveltje			0	25	90	100	
3. Diel vaj	0	15	60	95	100		
4. Dupuit asszony			0	15	80	95	
5. Favrené asszony	0	27	75	83	100		
6. Hardenpont téli vaj		0	15	40	95	100	
7. Nemes Krasszán	0	23	65	91	100		
8. Pap körte	0	20	60	100			
9. Pringalle vaj		0	10	40	90	100	
10. Serres Olivér	0	55	95	100			
11. Téli esperes	0	15	50	95	100		
12. Vilmos körte		0	4	15	80	100	

Table 3

Flower opening dynamics of pear varieties as expressed by the percentage proportion of flowers opening on subsequent days (1970)

Érd-Elvira

Variety	April						May				
	24	25	26	28	29	30	1	2	3	4	5
1. Bosc kobak			0	3	10	25	40	65	80	95	100
2. Clapp kedveltje			0	10	25	30	40	45	70	95	100
3. Diel vaj		0	5	95	100						
4. Dupuit asszony			0	5	20	25	40	45	65	80	100
5. Favrené asszony		0	5	55	80	85	90	100			
6. Hardenpont téli vaj		0	3	40	42	45	50	60	70	85	100
7. Nemes Krasszán		0	5	85	100						
8. Pap körte	0	3	30	90	93	95	97	100			
9. Pringalle vaj		0	2	30	55	60	67	70	95	100	
10. Serres Olivér	0	1	35	95	100						
11. Téli esperes			0	65	80	100					
12. Vilmos körte			0	40	50	65	70	80	85	95	100

Table 4

Heat amounts required for pear varieties to begin flowering, and trends in the average values of daily mean temperatures during flowering, in 1968—1970

Érd-Elvira

Year	Trend of flowering time in the pear variety collection	Heat amount required for the beginning of flowering (C°)	Average values of daily mean temperatures during flowering (C°)
1968	15—22 April	3491.1	14.85
1969	28 April— 2 May	3538.2	22.10
1970	24 April— 5 May	3502.3	19.33

methods of forecasting the beginning of flowering (Herbst—Rudloff 1939, Herbst—Weger 1940, Tamás 1959, Vitanov 1963, Anstey 1966, etc.).

According to the method elaborated by Herbst—Rudloff (1939) and Herbst—Weger (1940) 18 of the varieties required a heat amount of 3013—3440°C for the beginning of flowering on a five year avarage. On a six years' average Schossing (1959) found 12 pear varieties to begin flowering with a heat amount of 3435°C attained. In our investigations (Table 4) pear varieties began to flower when the heat amount attained an average of 3510°C (the lowest value was 3491°C in 1968, the highest 3538.2°C in 1969).

Fertility of pistils and dehiscence of anthers. The secretory activity of the pistils starts with a liquid drop appearing on the stigma when sexual maturity has been reached.

The fertility of the pistils is illustrated by Fig. 1. The figure shows that during the period of flower opening the pistils are fertile for a very short time and their "wilting" (browning and drying up) sets in rapidly. In selected flowers of the Bosc kobak clone No. 13/1 pistils were fertile for 2–3 days then wilted within 1–2 days.

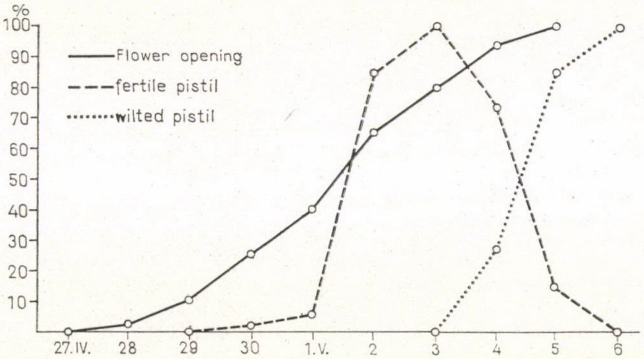

Fig. 1. Dynamics of flower opening and fertility of pistils. Bosc kobak 13/1 clone

In certain years (e.g. 1968) when at the time of blossoming the weather was dry and windy, the secretum drops of the pistils dried up within a single day. The maturing and dehiscence of the anthers as well as the dispersion of the pollen primarily depended on meteorological factors too. The dehiscence of the anthers within the same flower lasted for 1–2 days in hot, sunny weather (in 1969), and for 4–5 days in rainy, cool weather (in 1970). In the varieties examined sexual maturity of the pistils set in 1–4 days earlier than that of the anthers.

Conclusions

The possibility of cross-pollination depends on the coincidence of full bloom dates and the extent of simultaneous flowering. The best pollen donor varieties are the ones in which the time of pollen dispersion coincides with, or covers the time of pistil fertility in the receptor varieties. The method of Herbst—Weger (1940) has proved suitable for the prediction of the beginning of blossoming in the pear varieties.

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COMPENSATION OF YIELD COMPONENTS IN SPRING OATS AND THEIR SELECTION

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In an experiment of random block design, at a high level of soil fertility yield components of intensive varieties and strains of *Avena sativa* L. as well as their compensation under the condition of limited population density (216 panicles/m^2 on an average) were studied by linear regression analysis. The limited population density was used with the view of forcing the compensation mechanism of the yield components to function. A linear regression of positive correlation was found to exist between grain yield and panicle/m^2, grain yield and grain-number/panicle, grain yield and hl-weight, kg, and between grain yield and grain-straw ratio. With the exception of the last variant the regression correlation manifested itself at the lower range of the variation, therefore its breeding value was considered limited. This means that the highest values that show the regression correlation — i.e. the 270 panicles/m^2, the 85 grains/panicle and the 45 kg hl-weight — must by all means be attained during the selection, under the conditions of the experiment, otherwise the grain yield can be expected to decrease. This conclusion is not sufficiently perspective from the point of view of breeding. The situation is favourable in the case of grain yield and grain-straw ratio, as their regression is manifested in the upper range of the variation line thus giving a sufficient basis for selection. No regression correlation could be identified between grain yield and grain-weight/panicle, as well as between grain yield and thousand-grain-weight. As a consequence of limited population density grain weight per panicle increased to a great extent ($\overline{X} = 2.65$ g) and population deficiency was thereby compensated.

Introduction

The principle of "selection for yield" and the method of selecting on the basis of morphological yield components have been criticized in the last years. Selection made on the basis of yield components was considered by the authors to be of doubtful value because of their interaction and compensation mechanism.

Jones–Hayes (1967) studied for three years the influence exercised by the amount of seed rate on the grain yield and found that in the case of medium and high amounts of seed rate the effect of initial population difference was compensated in the later phases of development.

Degras (1964) carried out a detailed morphological yield analysis with spring oats. Accordingly it was only the weight of the panicle that could be used in selecting for yield increase.

Frey (1959) studied the effect of nitrogen fertilization on the yield components of oats, and by taking two components — panicle number/plot and grain number/panicle — jointly in consideration found it possible to screen the strains for N-fertilizer reaction. In the paper not only the averages of the individual components but their regression correlation with other components too were investigated by linear regression analysis. Under the extreme climatic conditions of Hungary a compensation ability which ensures high yields is both necessary and important.

Material and Method

The productivity of the spring oat varieties Condor, Romulus, Astor, of eleven spring oat strains obtained from the Institute of Pajbjergfonden and of the F-oat was studied in an experiment arranged in random block design with six replications. The experiment was carried out in a lowland region, on the alluvial soil of the river Maros, in the triangle formed by the rivers Tisza and Maros, at Kiszombor. The average fertilizer level was: 120 kg N, 60 kg P_2O_5 and 120 kg K_2O, a total of 300 kg active agent per ha. The size of each plot was 6 m × 0.5 m. The experiment was sown on March 27th 1971. Emergence was recorded on April 5th. In the regression analysis varieties and strains are represented by a single mean value. The regression analyses were performed by the JATE Cybernetic Laboratory, Szeged.

Results

By means of linear regression analyses correlations were found between the grain yield on one hand, and panicle number/m², grain-number/panicle, hl-weight and grain-straw ratio, on the other. No regression correlation could be identified, however, between grain yield and grain-weight/panicle, nor between grain yield and thousand-grain-weight (Table 1).

Table 1

Table of correlations

Characteristics	$\bar{x}$	2	3	4	5	6	7	8
1. Grain yield/m² (dkg)	57.3	+	0	+	0	+	+	+
2. Panicle number/m²	216		—	0	—	0	0	0
3. Grain-weight/panicle, g	2.65			+	+	0	0	0
4. Grain-number/panicle	89				—	0	0	0
5. Thousand-grain-weight, g	29.6					0	0	0
6. Hl-weight, kg	45.6						0	+
7. Grain-straw ratio	0.73							0
8. Grain-husk ratio	2.42							

+ positive correlation
— negative correlation
0 no correlation

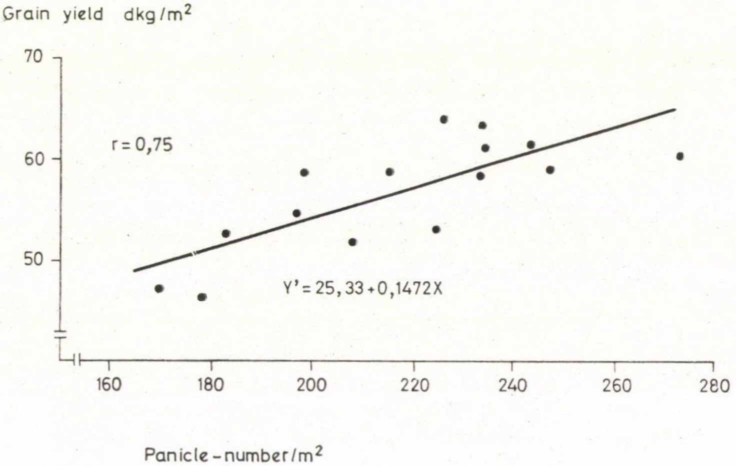

Fig. 1. Correlation between grain yield and panicle number

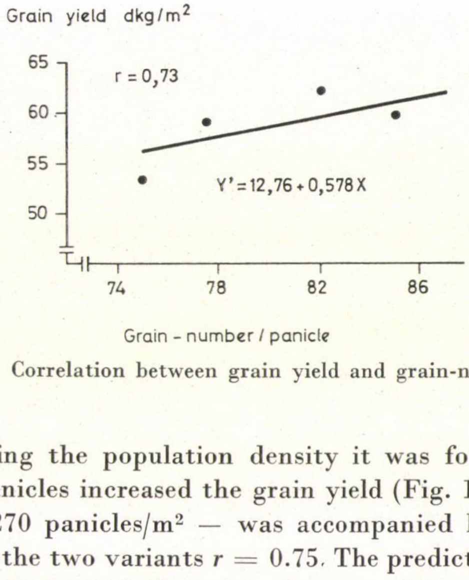

Fig. 2. Correlation between grain yield and grain-number/panicle

On examining the population density it was found that a growth in the number of panicles increased the grain yield (Fig. 1). The highest population density — 270 panicles/m^2 — was accompanied by a grain yield of 65 dkg/m^2. Between the two variants $r = 0.75$. The predicted effect of the changing stand is very high: 7.35 dkg/m^2 in the case of 50 panicles.

No general linear regression is found between the grain yield and the number of grains per panicle. Such correlation could be observed only in the 75—85 grain-number/panicle interval (Fig. 2), where the effect is considerable and $r = 0.73$.

Linear regression was shown between grain yield and 42—44.4 kg hl-weight; $r = 0.94$, $b = 4.5$ dkg/hl-weight kg (Fig. 3). No regression correlation of grain yield to hl-weight was experienced when the hl-weight was further increased.

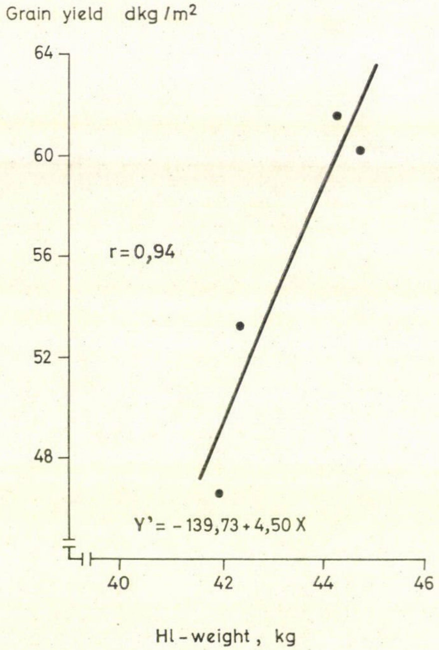

Fig. 3. Correlation between grain yield and hl-weight

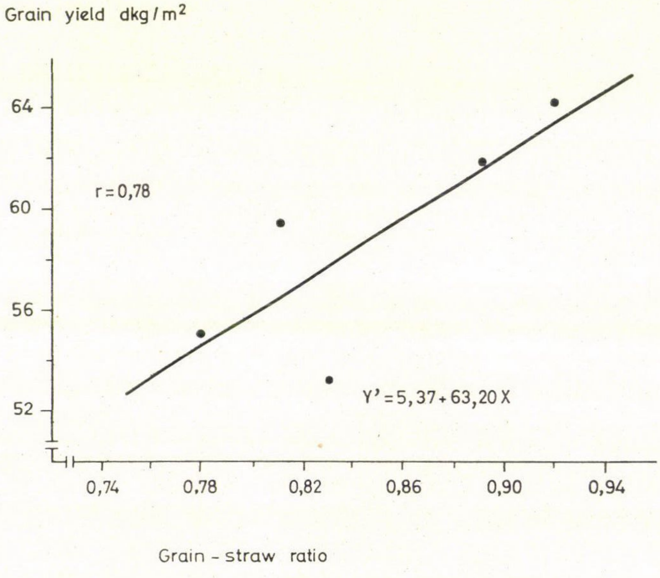

Fig. 4. Correlation between grain yield and grain-straw ratio

In the 0.78—0.91 grain-straw ratio interval $r = 0.78$ between the grain yield and the grain-straw ratio. When the grain-straw ratio increased by 0.1 an improvement of 6.3 q/ha grain yield could be estimated (Fig. 4).

As for the correlation between grain yield and grain-husk ratio the correlation coefficient was 0.50.

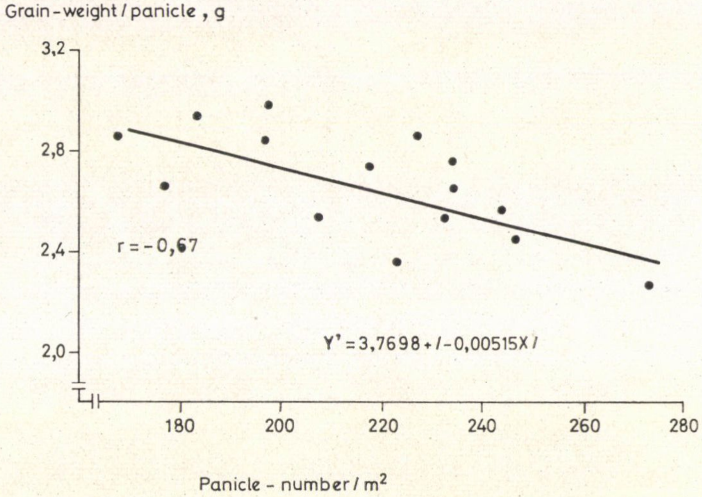

Fig. 5. Correlation between grain-weight/panicle and panicle-number/m^2

No regression correlation could be pointed out between the grain yield and grain-weight/panicle, nor between grain yield and thousand-grain-weight. The phenomenon is caused in essentials by the function of the compensation mechanism. The development of a correlation between grain yield and grain weight per panicle was partly prevented by the negative correlation between grain-weight /panicle and panicle number/m^2 (Fig. 5).

With the increase of panicle number/m^2 the grain weight per panicle considerably decreased. In the correlation $r = 0.67$.

A linear regression of positive correlation was found between grain number/panicle and grain-weight/panicle (Fig. 6): $r = 0.72$.

When the thousand-grain-weight increased from 26 g to 32 g the grain weight per panicle increased too: $r = 0.65$ (Fig. 7). Finally, Fig. 8 shows an increase of the hl-weight expected with the improvement of the grain-husk ratio: $r = 0.78$.

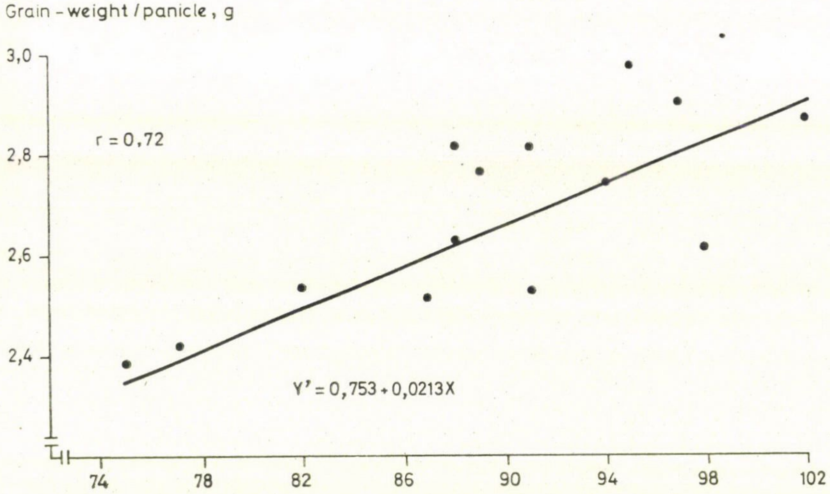

Fig. 6. **Correlation between grain-weight/panicle and grain-number/panicle**

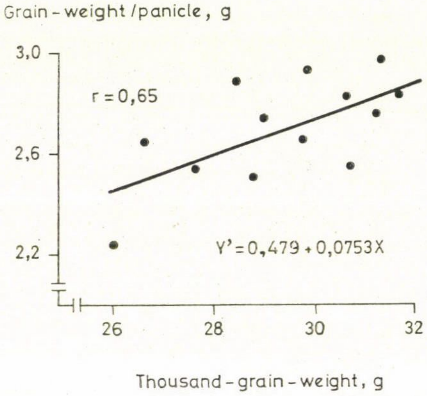

Fig. 7. **Correlation between grain-weight/panicle and thousand-grain-weight**

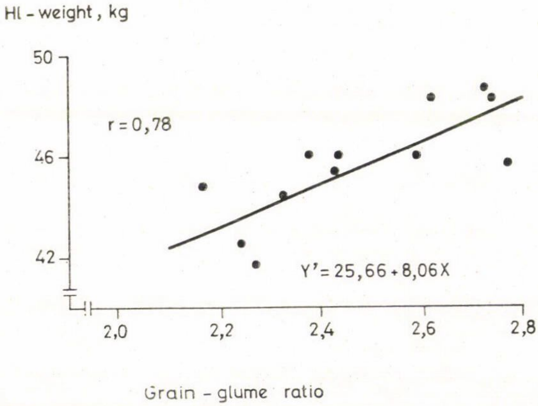

Fig. 8. **Correlation between hl-weight and grain-husk ratio**

Conclusions

When drawing conclusions we must keep the following circumstances in mind:

a) the experiments were performed with highly productive, so called intensive varieties and strains;

b) soil fertility was increased by adding an average of 300 kg/ha active agent;

c) population density was limited to a level which, in interaction with genotype and soil fertility, made possible the function of the compensation mechanism of yield components, and at the same time a high level of yield. (The average yield of 90 plots was 57.3 dkg/m^2.)

These circumstances ensured that the linear regression of characteristics related with the grain yield could be demonstrated.

The population density of 270 panicles/m^2 is considered to be a marginal condition under which, in the case of an effective compensation, an outstanding grain yield (65 dkg/m^2) can even be attained and, at the same time, a certain degree of selection made for the yield components and compensation ability. In our further experiments we intend to increase the population density — expressed by the index panicle/m^2 — to a limit where it is still accompanied by increased yields, in order to be able to study the yield components and the compensation mechanism at that level too.

As a result of compensation no regression correlation was found between grain yield and grain weight per panicle. In spite of this fact we deem it important to emphasize its effect on the yield. As a consequence of limited population density the per panicle grain weight considerably increased in all varieties and strains and attained an average value of 2.65 g/panicle. Population deficiency was compensated by an increased grain weight per panicle; it was in this that the effects of grain number and grain weight were added up.

When varying the per panicle number of grains from 75 to 101, linear regression of positive correlation was found only with grain/panicle values between 75 and 85. It was remarkable that the regression was manifested only in the lower range of the variation line. This means that under the conditions of the experiment 85 grains per panicle must by all means be attained. However, this does not even represent the average and is not sufficient for the compensation.

A growth in the number of grains per panicle increased the per panicle weight of the grains and decreased the thousand-grain-weight. After all, no correlation was found either between grain yield and grain-weight/panicle, or between grain yield and thousand-grain-weight.

In the regression correlation between grain yield and 42—44.4 kg hl-weight the values of r and b are very important, therefore a minimum

44—45 kg hl-weight has to be ensured. However, this is not sufficient to attain high yields and adequate compensation. In this case too, regression is manifested with the low values within the range of variation, and even the highest value (44.4) involved in the regression is below the average (45.6) hl-weight.

The increase of grain-straw ratio in the interval of 0.78—0.91 was also connected with an increase in yield. Increasing the grain yield or decreasing the straw yield in order to improve the ratio are equally justified. One of the components in the grain-straw ratio is grain itself, therefore the regression of both grain yield and grain-straw ratio is manifested in the upper range of variation. Regression correlations between grain yield on one hand, and panicle number/m^2, grain-number/panicle and hl-weight on the other, were found to be manifested in the lower range of variation. Therefore they show a minimum value which must by all means be attained during the selection, otherwise a decrease in the grain yield can be expected. They have, thus, a limited selection value. It is above these values that the compensation mechanism functions.

The above results suggest that in breeding for yield increase it is more important to know and improve the compensation ability of yield components than to know the regression of the characteristics.

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INFLUENCE OF DIFFERENT TOP-SOIL MOISTURES ON NITROGEN AND PHOSPHORUS UPTAKE BY MAIZE

By

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A greenhouse experiment was conducted to determine the influence of different top-soil moisture contents on the uptake of N and P from fertilizer by maize, when the lower part of the roots were in water or in water containing N and P fertilizers. The data show that the soil moisture level had a highly significant influence on the N and a fairly small effect on the P content of the plants. The effect of soil moisture on the % N and P derived from the fertilizer was very significant. P uptake from the fertilizer added to the soil was quite small when the plants were supplied with water by the lower part of their roots. The N content of the plant derived from the fertilizer was relatively high from dry soil, though it was 2–5 times less compared to the treatment with moist soil. The soil moisture content had no significant influence on the ^{15}N uptake from the solution but the P uptake from the solution increased with a decreasing soil moisture level. In the treatments where NP were supplied both to soil and water, the ^{15}N uptake was not significantly affected by the soil moisture whereas the ^{32}P uptake was higher for a lower moisture level.

Introduction

All the life processes of a plant are significantly influenced by soil moisture. Many experiments (Debreczeni *et al.* 1968, Mederski—Wilson 1960, Olson *et al.* 1961, Power—Grunes 1961, Sabinin 1940) have shown that above the wilting point if the soil moisture content is increased, the growth and nutrient uptake of plants also increase. However, the nutrient content of the plants is usually not influenced linearly by the decrease in soil moisture content (Fawcett—Qutrk 1962, Petinov 1965, Peterburgskiy 1959, Show 1955). The opinions on nutrient uptake by plants from different moist layers are contradictory. According to Breaezeale (1930), Smirnov (1958) and Hunter—Kelley (1946), plants can take up nutrients from dry soil, when a part of their root system is in a moist layer and in this way have been supplied with water. The nutrient uptake then takes place from a moist microzone, formed around the roots in the dry soil. According to others (Koritzkaya 1939, Sokolov 1946), nutrient uptake from dry soil is small and depends on the plant species, the nature of the nutrient elements, their concentration and on the physical-chemical properties of the soil.

To throw more light on this problem a pot experiment was carried out using labelled N and P fertilizers. The objectives of this experiment were to determine: *a)* the NP uptake from soils of different moisture content when the lower parts of the roots were in water; *b)* the NP uptake from a solution, when the upper parts of the roots were in soils of different moisture content; *c)* the NP uptake, when 50% of the NP was supplied in the soil, and the other 50% in a water culture.

Material and Method

The soil used in this experiment was sandy loam with a water holding capacity of 30% and a wilting point of 23% of the water holding capacity. The pF was 4.1.

The pots consisted of two parts. The upper part was a plastic tube with a metal sieve at the bottom, and the lower part a 600 ml beaker. The upper part contained soil, and the lower part water.

Between the soil and the water a thin paraffin layer was coated on the screen to prevent the movement of water from the lower part of the pot into the soil.

The paraffin layer on the metal sieve was about 2 mm thick. A few particles of vermiculite were placed on the paraffin layer.

The quantity of dry soil in each pot was 150 g and the soil layer was about 10 cm high.

Two tubes which had holes in the sides, were put into the soil for water supply. After the seeding of the maize, the soil was moistened to 74% of the water holding capacity. The nutrient solution was added to each beaker and the solution layer was kept at a height of

Table 1

Dry matter yield and NP uptake by maize

Treatment					Dry matter weight per pot in grams	Plant composition			
Average soil moisture in percentage				Method of application of the ^{15}N and ^{32}P labelled fertilizers		% N	% N derived from fertilizer	% P	% P derived from fertilizer
of water holding capacity		of dry weight							
Before NP application	At harvest	Before NP application	At harvest						
74	72	22.0	22.6	In soil	7.45	2.01	9.27	0.36	2.30
				In water	5.80	2.15	21.04	0.42	11.40
				In soil and water (1:1)	6.18	1.95	10.23	0.37	7.46
30	27	9.0	8.2	In soil	6.40	1.66	5.56	0.34	0.09
				In water	6.00	2.01	18.65	0.39	17.14
				In soil and water (1:1)	6.45	1.68	9.69	0.36	11.30
21	21	6.3	6.3	In soil	8.21	1.57	2.06	0.34	0.07
				In water	6.69	1.79	19.92	0.40	17.90
				In soil and water (1:1)	5.85	1.66	11.66	0.38	10.00

20 cm. During the first stage of plant development the water level in the beakers, and the moisture content of the soil were kept at the same level until the labelled fertilizer was applied. 3 plants were grown in every pot. During the growing time the water was continuously aerated.

The water supply was stopped in treatments (b) and (c) 38 days after seeding, when the plant height was about 30 cm.

Four days later, when the soil was dry, the moisture level of each pot was determined. Then the soil in the pots of treatment (b) was brought to a moisture content of 27% of the water holding capacity. The pots of treatment (c) did not receive any moisture.

At the same time equal amounts of NP were applied to all treatments at a rate of 45 mg N and 15.6 mg P per pot. N was applied as $(NH_4)_2SO_4$ with an atom excess of 5% ^{15}N and P in the form of KH_2PO_4, labelled ^{32}P. The nutrients were mixed with sand and applied in 4 holes. In the lower parts of the pots the nutrient solution was replaced by water to which 10 ml labelled NP solution were added. The experiment was carried out with 3 replications.

45 days after seeding — 3 days after NP application — the maize was harvested, and separated into roots and tops. The tops were dried at 70°C, weighed, finely ground and analysed for nitrogen content and $^{14}N/^{15}N$ ratio. Subsamples were taken for the analysis of phosphorus and ^{32}P content. The total N content was determined on the basis of Kjeldahl digestion (JACKSON 1958). ^{15}N was determined by mass spectrometry (MERSARI—BROESHART 1968). Phosphorus was determined colorimetrically and the ^{32}P content by a Nuclear-Chicago liquid scintillation detector.

The soil moisture content, before the fertilizer application and at harvest time was determined gravimetrically (Table 1).

Results

The dry matter production, the total N and P contents and fractions derived from the applied fertilizers are given in Table 1.

The differences in the yield data show a rather high variation which could not be related to the differences in moisture content after 38 days. With reduced soil moisture content, the total and % N uptake from the fertilizer decreased. In those treatments where the nutrients were put in water, the % N and P were the highest.

The quantity of N derived from the fertilizer being applied to the soil clearly shows the positive relationship between the N uptake and the soil moisture content. Furthermore, essentially P was not taken up by the plants from fertilizers applied to the soil which had a moisture content near to wilting point, even though the lower part of their roots were in standing water.

When the labelled P fertilizer was added to water, the P derived from the fertilizer in the water was higher in the plants growing in the soil with a lower moisture content, reaching a value of 17.9 and 17.1% for the lower soil moisture levels as compared to 11.4% for the highest soil moisture level. In those treatments where N was added to the water distributed in soil and water there were significant differences in fertilizer N uptake. The plants were readily supplied with nutrients from the solution. However, from dry soil the relative uptake of fertilizer N was greater than that of fertilizer P.

The data for the mixed application show that the uptake of N and P from the fertilizer were higher than when the fertilizers were applied to the soil.

It was assumed that the small variation in the soil moisture content during the 3 days between fertilizer application and harvest had no important effect on the water supply of the plants.

Summarizing, it may be stated that the experiment showed that nutrient uptake takes place when an adequate water supply is present around the root system. Apparently in dry soil no sufficient water transport takes place through the roots and the moistening of the microzones around the dry roots when a part of the roots are adequately supplied with moisture.

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STUDIES ON ABNORMAL GROWTH IN PLANTS

II. STRUCTURE OF CAULINE TUMOURS IN PROSOPIS SPICIGERA L. INDUCED BY INSECTS

By

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The present study includes the anatomy of cauline galls of *Prosopis spicigera* induced by insects. The infection by the insect takes place at the early stages of development of the stem. In the cauline galls, the insects are discernable in the cortical parenchyma and in the medullary rays. The infected stem shows an accelerated production of wood. The formation of vascular rays is stopped due to infection. The production of a larger amount of apotracheal parenchyma interspersed between the tracheidal groups and the formation of lysigenous cavities are the other features noted in the development of cauline galls.

Introduction

Prosopis spicigera L., the plant under present investigation, is one of the few trees which thrive well in the semi-arid regions of Northern India. The leaves of this plant are extensively used as fodder and the wood as firewood. The tender fruits are a delicacy as vegetable in some parts of Rajasthan. However, the plants are invariably infected by insects; the leaves and flowers by *Eriophyes prosopidis* Saksena and the petioles and stem by an unknown chalcid insect (MANI 1964). The malignant infection by these insects adversely affects the growth and reproduction of the tree as the infested organs are converted into tumors of various shapes and sizes.

In a previous communication VARGHESE—SHARMA (1971) studied the developmental anatomy of foliar and petiolar galls in *Prosopis spicigera*. SHARMA—VARMA—VARGHESE (1972) described the anatomy of floral galls in *Prosopis spicigera* L. The present study records the anatomy of cauline galls in this plant.

Material and Method

The material used for the present study was collected from Hissar (India) and includes normal stem parts of *Prosopis spicigera* and galls produced on them. The cauline galls were collected in the months of May, June, July, August and December. The materials collected at different stages of maturity were fixed in formalin–acetic acid–alcohol (FAA). Transverse and longitudinal sections were cut 8 to 15 μ thick and stained with safranin–fast green combination by the usual method (JOHANSEN, 1940).

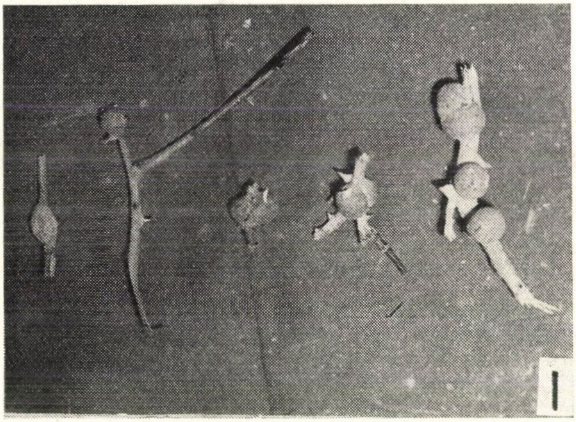

Fig. 1. Developmental stages of cauline tumours. ×127

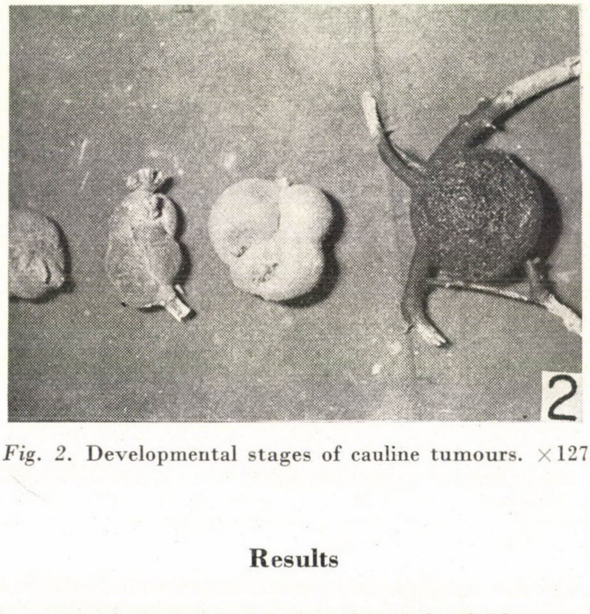

Fig. 2. Developmental stages of cauline tumours. ×127

Results

Stem galls start their initiation at the end of May and take at least one year to attain full stage. These are light brown in colour and delicate to start with, but become hard towards the end. To begin with, the cauline galls are globular, oval or pipette-shaped. More than one gall may be formed from a small branch either conjointly or separately (Fig. 1). The cauline galls may be asymmetric if the growth is only on one side or symmetric if the growth is radial. The mature gall is either lobed or globular. Formation of a number of branches diverging from the gall due to the suppression of internodes is a usual feature (Fig. 2). The bark is peeled off from a fully grown

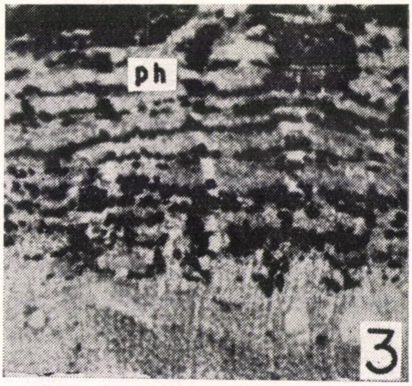

Fig. 3. A portion of the transection of a normal stem (ph, phloem). ×127

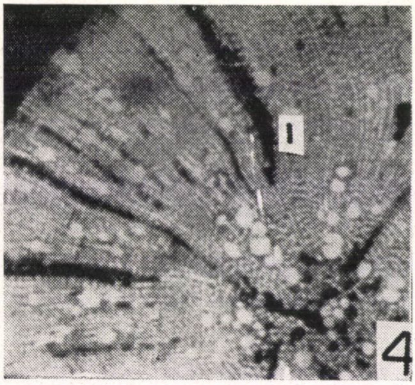

Fig. 4. Transection of a young gall with larvae inside the medullary rays (l, larvae). ×127

gall exposing a number of pores each forming an outlet for the insect, or providing fresh air to the insects inside (Fig. 2). The branches above the gall may continue their growth for some time or may dry up. The reason for such a behaviour of stem galls is discussed elsewhere in this paper.

A normal stem which has undergone secondary growth shows an elaborate production of cork cells. The phellogen and phelloderm cannot be distinguished. However, below the bark there is a layer which consists of dark cells. At this region there are 2 to 3 layers of parenchymatous cells arranged in regular tiers with the stone cells interspersed in between. The secondary phloem is present in discrete units separated by a single layer of parenchymatous cells filled with tannin. The single concentric layer of phloem represents the formation of the phloem in a single season (Fig. 3). Most of the cells of the phloem are converted into phloem fibres while the rest remain as parenchymatous cells. The growth rings are very clear in the secondary xylem. It is obvious that the growth in thickness of the stem is extremely slow and takes several years to increase even a few centimeters in diameter. The wood is dissected by radially running uni- or biseriate vascular rays (Fig. 4). The vessel elements are dispersed between the rays. The vessel groups are normally surrounded by a limited amount of vasicentric paratracheal parenchyma. The amount of parenchyma in a gall is less then the tracheids in a normal stem. The pith is present in the central portion and consists of isodiametric parenchymatous cells. The infection by the insects takes place when the stem is two to three years old as indicated by the number of growth rings. The insects enter in the bark at this stage and move through the periphery first, and then radially through the medullary rays (Fig. 4). A large number of insects could be noted in the vascular rays. In certain instances more than one insect was found in a single ray-cell (Fig. 5). The infested stem shows remarkable devia-

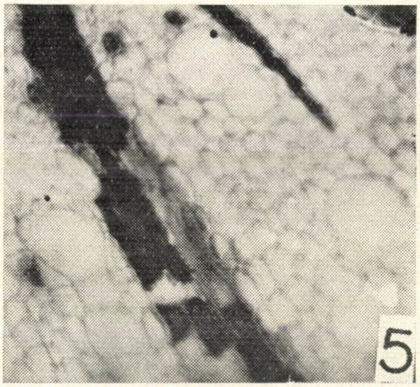

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Fig. 5. Enlarged medullary rays with insect larvae inside. ×621

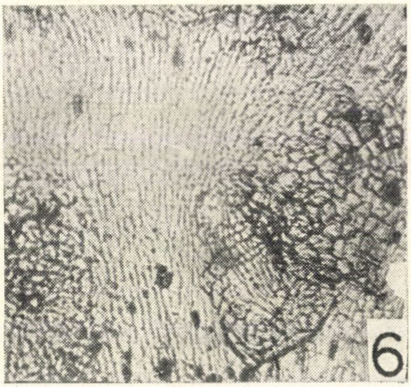

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Fig. 6. A portion of the gall showing excessive production. ×621

tion from the normal stem in the development of the secondary tissues. Each cambial layer functions for a longer duration than in the normal stem and forms parenchymatous cells corresponding to phloem cells towards the outer side at a rapid rate. These cells instead of getting converted into phloem mature into parenchymatous cells. Although in the beginning these cells are arranged in regular tiers, at later stages due to shortage of space and excessive production of parenchyma the regularity in their arrangement is disturbed. Some of the cells develop thick walls and remain in groups, while others possess dark contents (Fig. 6). These cells are normally scattered or remain in small groups. The formation of vessels is much different from that of a normal stem, especially in size, shape of the constituent cells and also in the amount of elements produced. It is noted that the infected plants in one season produce several times more wood than the normal ones. An excessive production of tracheids and parenchymatous cells and the absence of vascular rays are the features of interest in the infected stem. In the infected stem a large amount of parenchyma is produced which remains apotracheal and interspersed between the tracheidal groups which form either a complete or broken ring around the parenchyma. The eggs of insects are deposited in the wood parenchyma cells. The insects hatched from these eggs are located in the cortical parenchymatous cells and in medullary rays. In the former these insects are located periclinally and in the latter radially. The disintegration of these cells form lysigenously originated cavities (Fig. 7). In the stem which is induced to produce the gall, the tracheids differentiate in a radial direction in contrast to the longitudinal differentiation in a normal stem. These vessels instead of forming longitudinal series show a ring-like appearance. Each ring is composed of a number of vessel elements. Vessels in a gall often give a zig-zag appearance or resemble the shape of a crawling snake (Fig. 8). Develop-

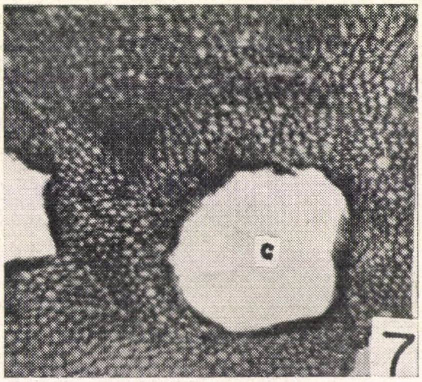

Fig. 7. Lysigenous cavities inside the gall (C, cavity). ×621

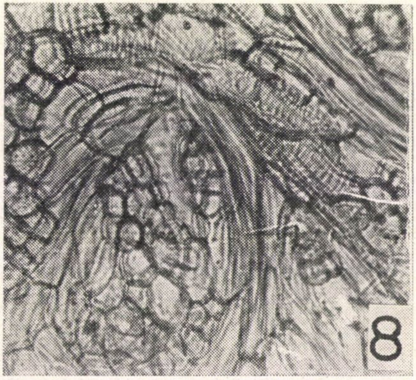

Fig. 8. The tracheids formed after infection showing the changed pattern. ×621

ment of spirally arranged vessel groups is also not uncommon (Fig. 8). In the former, in between the vessels, the tracheids may assume the shape of multiseriate or uniseriate rays (Fig. 8). These tracheidal elements differ from the normal tracheids in having an isodiametric shape. The end walls of transversely arranged vessels instead of remaining straight become oblique, thus resembling the end wall of a tracheid.

Discussion

The development of stem galls in *Prosopis* presents certain interesting features. The infested stem portion responses distinctly to secondary growth. The growth rings in a normal stem are very narrow, whereas in an infected stem they show an increased production of various types of cells by the secondary cambium. The cells produced towards the periphery, instead of getting converted into phloem cells, mature into parenchyma. Another feature which is of significance is the complete cessation of the production of medullary rays which are extremely prominent in a normal stem. In place of medullary rays, groups of parenchymatous cells are formed.

In *Prosopis spicigera* there is an excessive production of apotracheal parenchyma in the abnormal stem. The tracheidal elements are also produced in large amounts. The pattern of development of the vessel elements is also changed considerably. The initials of vascular elements, instead of developing into longitudinal series, develop in an oblique, transverse or spiral series.

The distribution of insects inside the stem provides a curious instance. The insects in the stem are always visible in the parenchymatous cells of the cortical region arranged in a periclinal direction and in the vascular rays in a radial direction. This special mode of distribution provides an interesting instance as it provides least resistance for these insects to move in or out when the cauline gall matures as the pores develop at the ends of the vascular rays facing the cortical tissue.

The biological association of the insects in the stem of *Prosopis* is in no way beneficial to the plant but brings about severe detrimental consequences. The production of cauline galls causes the shortening of the axis and the approximation of the nodes and, in extreme cases, the death of the branches produced above the level of the galls.

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VARIETAL AND NITROGEN EFFECTS ON DOUGH CHARACTERS AND PROTEIN CONTENT OF SOME NEW HIGH YIELDING WHEAT VARIETIES

By

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Nine recently developed high yielding wheat varieties grown under three levels of nitrogen fertilization were tested for protein content and dough characteristics such as dough development time, stability, mixogram area, elasticity, dough development angle and peak height. The results show that varietal differences were significant for all the characters whereas effects due to nitrogen were significant only for peak height, elasticity and protein percentage. Considering the relative dough characteristics of the different varieties, Heera, HD.1944 and HD.1949 have come out very promising for dough development time, stability and mixogram area, the three characters particularly important from the standpoint of baking quality. Correlation studies showed that dough characters are not influenced by protein content.

Introduction

Recent studies conducted with a number of new high yielding wheat varieties showed highly significant increases in the protein content of the grain due to nitrogen fertilization and that varietal differences with regard to their response to the above treatment were also highly significant (Austin—Miri 1961, Austin—Kumar—Nair 1971). Beneficial effects of nitrogen fertilization on the protein content in wheat grain were reported earlier by several workers (Halliday 1960, Pfaff 1955, Linser 1950, Primost 1960). However, information on the effect of nitrogen fertilization on physical dough characters are rather scanty. Since the modern improved wheat varieties have been found to differ significantly among themselves for dough characters, studies have been initiated to determine as to what extent the dough characteristics are typical of a variety and how far these are influenced by nitrogen fertilization. Information on these aspects is very useful to plan wheat production on the basis of quality particularly with reference to the special requirements of the baking industry.

Material and Method

The grains of nine recently evolved high yielding varieties raised under three levels of nitrogen namely, 0, 120 and 200 kg N/ha were tested. The samples were milled in a Buhler automatic flour mill and the straight-run flour obtained was tested for dough characters using a mixogram. 30 g flour and 21 ml water were used for each test. From the streaked diagram (graph) obtained, characters such as dough development time, stability, peak height, elasticity, angle of dough development and area of the curve were measured as indicated in Fig. 1.

Protein (N × 5.7) content was estimated according to the usual macro-Kjeldahl method using 0.5 g flour for each test.

Statistical analysis: The data for the various characters obtained with composite samples were analyzed by using a two way tested classification. The effects of varieties and levels were tested in the usual manner using the variance ratio, interaction being used as error. The appropriate critical differences and bar diagrams are presented.

Results

The analysis of variance presented in Table 1 shows that varietal differences were significant at 1 per cent level for all the characters except protein percentage which was significant at 5 per cent level. Differences owing to nitrogen were found to be significant for peak height, elasticity and protein percentage.

The data for the different dough characters and protein content owing to variety and treatment differences are presented in Tables 2 and 3 respectively. It is seen that the values for dough development time showed significant varietal differences. The mean values due to variety averaged over the effects of treatment varied from 1.83 min. to 4.66 min. The highest dough development time was given by Heera, a high yielding triple gene dwarf variety recently released for commercial cultivation. This variety came out signifi-

Table 1

Analysis of variance

	Dough time	Development		Stability		Peak height	
	D. F.	*M. S.*	*F.*	*M. S.*	*F.*	*M. S.*	*F.*
Varieties	8	1.994	11.445**	0.784	12.957**	19.898	3.961**
Nitrogen	2	0.377	N. S.	0.225	N. S.	65.148	12.969**
Error	16	0.174		0.060		5.023	

	Elasticity		Dough development angle		Mixogram area		Protein	
	M. S.	*F.*	*M. S.*	*F.*	*M. S.*	*F.*	*M. S.*	*F.*
Varieties	8.280	6.468**	403.870	11.880**	6.829	17.362**	1.529	2.951*
Nitrogen	10.260	8.014**	87.370	2.570 N. S.	1.890	4.805 N. S.	7.147	13.796**
Error	1.280		33.995		0.393		0.518	

cantly superior to all the varieties. The lowest value was given by HD. 1539 which was at par with 3 other varieties namely D. 2117, Kalyansona and Pusa Lerma.

As for dough stability, the mean values due to variety averaged over the effects of nitrogen varied from 0.58 to 2.18 min. HD. 1944 showed the highest value. Heera, EA. 222-1 and HD. 1949 were at par with each other. The stability was lowest in D. 2117. This variety was at par with HD. 1539 which again was at par with Pusa Lerma.

Heera with a mean value of 9.07 cm^2 for mixogram area came out significantly superior to the rest of the varieties. The lowest value of 4.50 cm^2 was found in HD. 1537 and this variety was at par with 4 other varieties namely D. 2117, EA. 222-1, Pusa Lerma and HD. 1674. It may be noted that varieties such as Heera, HD. 1944, HD. 1674, EA. 222-1 and HD. 1949 in general had come out superior to the rest of the varieties for mixing time, stability and mixogram area. Kalyansona, although was similar to Pusa Lerma and D. 2117 for mixing time, was significantly superior to the above two varieties for stability and mixogram area.

The significantly highest value of 14 mm for elasticity was found in HD. 1944. With the values varying between 8.67 to 11.33 mm the rest of the varieties namely D. 2117, HD. 1539, Heera, HD. 1949, HD. 1674 and EA. 222-1 were at par with Kalyansona or Pusa Lerma. The data for angle of dough development show a range of variation from 36° to 75°. Varieties such as HD. 1539, D. 2117, Kalyansoan and Pusa Lerma which had given low values for dough development time had shown higher values for angle of dough development. Conversely, varieties such as Heera, HD. 1944, HD. 1674, EA. 222-1 and HD. 1949 having relatively higher dough development time had lower angle of dough development.

The peak height of the mixogram varied from 38.00 mm to 44.33 mm. The varietal differences were significant. It may be noted that varieties (such as Heera, Pusa Lerma, etc.) which differed markedly among themselves for the various characters particularly dough development time, stability, angle of dough development and mixogram area, had more or less similar values for peak height. It appears that this character cannot be taken as an indicator for the discrimination of varieties.

The protein content in these varieties varied from 11.93 to 14.36 per cent. The varietal differences were highly significant. The highest value of 14.36 per cent was found in Pusa Lerma, an amber grain mutant developed at the Indian Agricultural Research Institute from the red-grained high yielding Mexican dwarf variety Lerma Rojo.

The importance of dough characters in determining bread making quality had been pointed out by several workers. According to SWANSON (1943), JOHNSON *et al.* (1943) and SHUEY—GILLES (1966), some of the desir-

Table 2

The values for the various dough characters due to varieties and nitrogen levels

Variety	Character	Nitrogen levels			Mean due to variety
		N_0	N_{120}	N_{200}	
Heera	Mixing time	4.50	5.00	4.50	4.66
	Stability	2.00	2.00	2.00	2.00
	Mixogram area	9.00	9.10	9.10	9.07
	Elasticity	11.00	11.00	11.00	11.00
	Dough development angle	35.00	35.00	38.00	36.00
	Peak height	35.00	39.00	40.00	38.00
	Protein	12.23	12.61	14.13	12.99
HD. 1949	Mixing time	3.00	3.50	2.75	3.03
	Stability	2.00	1.25	2.00	1.75
	Mixogram area	6.30	6.40	6.50	6.40
	Elasticity	9.00	10.00	9.00	9.33
	Dough development angle	53.00	45.00	55.00	51.00
	Peak height	40.00	40.00	38.00	39.33
	Protein	12.02	13.00	13.20	12.74
HD. 1674	Mixing time	3.50	3.50	3.00	3.33
	Stability	1.75	1.75	1.25	1.58
	Mixogram area	3.50	6.10	6.90	5.50
	Elasticity	7.00	9.00	10.00	8.67
	Dough development angle	43.00	48.00	55.00	48.76
	Peak height	35.00	43.00	45.00	41.00
	Protein	11.52	13.16	13.25	12.64
EA. 222-1	Mixing time	2.25	4.00	3.50	3.25
	Stability	2.00	1.50	2.00	1.83
	Mixogram area	4.50	5.30	5.70	5.10
	Elasticity	7.00	10.00	9.00	8.67
	Dough development angle	59.00	40.00	48.00	49.00
	Peak height	36.00	40.00	38.00	38.00
	Protein	11.73	12.36	12.78	12.29
HD. 1944	Mixing time	3.25	3.75	3.50	2.50
	Stability	2.25	2.00	2.00	2.08
	Mixogram area	6.60	8.20	8.50	7.77
	Elasticity	12.00	15.00	15.00	14.00

Table 2. (cont.)

Variety	Character	Nitrogen levels			Mean due to variety
		N_0	N_{120}	N_{200}	
	Dough development angle	50.00	49.00	50.00	49.67
	Peak height	40.00	45.00	45.00	45.33
	Protein	12.32	12.61	12.70	12.54
HD. 1539	Mixing time	2.00	1.75	1.75	1.83
	Stability	1.00	1.00	0.50	0.83
	Mixogram area	4.50	4.00	5.00	4.50
	Elasticity	9.50	10.00	14.00	11.17
	Dough development angle	71.00	75.00	79.00	75.00
	Peak height	40.00	43.00	50.00	44.33
	Protein	11.94	13.33	15.06	13.44
Pusa Lerma	Mixing time	3.00	2.50	2.00	2.50
	Stability	1.00	1.00	1.25	1.08
	Mixogram area	5.00	5.70	5.40	5.37
	Elasticity	8.00	10.00	12.00	10.00
	Dough development angle	49.00	60.00	70.00	59.67
	Peak height	34.00	40.00	43.00	39.00
	Protein	11.77	14.35	16.45	14.36
Kalyansona	Mixing time	2.50	3.00	2.00	2.50
	Stability	2.00	1.25	1.50	1.58
	Mixogram area	7.30	7.10	7.20	7.20
	Elasticity	10.50	9.00	11.00	10.17
	Dough development angle	62.00	58.00	72.00	64.00
	Peak height	42.00	45.00	45.00	44.00
	Protein	13.50	12.11	12.38	11.93
D. 2117	Mixing time	2.50	2.25	2.75	2.50
	Stability	1.00	0.50	0.50	0.59
	Mixogram area	4.80	5.00	5.30	5.03
	Elasticity	10.00	12.00	12.00	11.33
	Dough development angle	61.00	70.00	63.00	66.00
	Peak height	40.00	45.00	43.00	42.66
	Protein	12.91	13.08	13.75	13.25

C.D. for varietal means: 1. 0.72; 2. 0.42; 3. 1.09; 4. 1.96; 5. 10.09; 6. 3.88; and 7. 1.24

Table 3

Mean values due to nitrogen averaged over the effects of varieties

Characters	Nitrogen levels		
	N_0	N_{120}	N_{200}
Mixing time	3.55	3.36	2.86
Stability	1.67	1.36	1.44
Mixogram area	5.72	6.32	6.62
Elasticity	9.33	10.66	11.44
Dough development angle	53.67	53.33	58.89
Peak height	38.00	5.22	43.00
Protein	11.97	13.01	13.74

C.D. for nitrogen levels: (1) N, S; (2) N. S.; (3) N. S.; (4) 1.12; (5) N. S.; (6) 2.23. and (7) 0.74

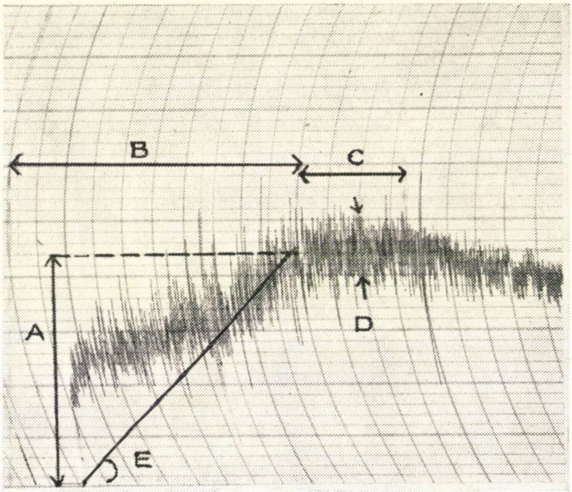

Fig. 1. Mixogram characters. (A = Peak height; B = Dough development; C = Stability; D = Elasticity; E = Angle of dough development)

able dough characteristics from the standpoint of bread making are high mixing tolerance, longer dough developmental period and higher values for the width and area of the curve. Considering the relative dough characteristics of the different varieties, Heera, HD. 1944 and HD. 1949 have come out particularly promising for characters such as dough development time, stability and mixogram area.

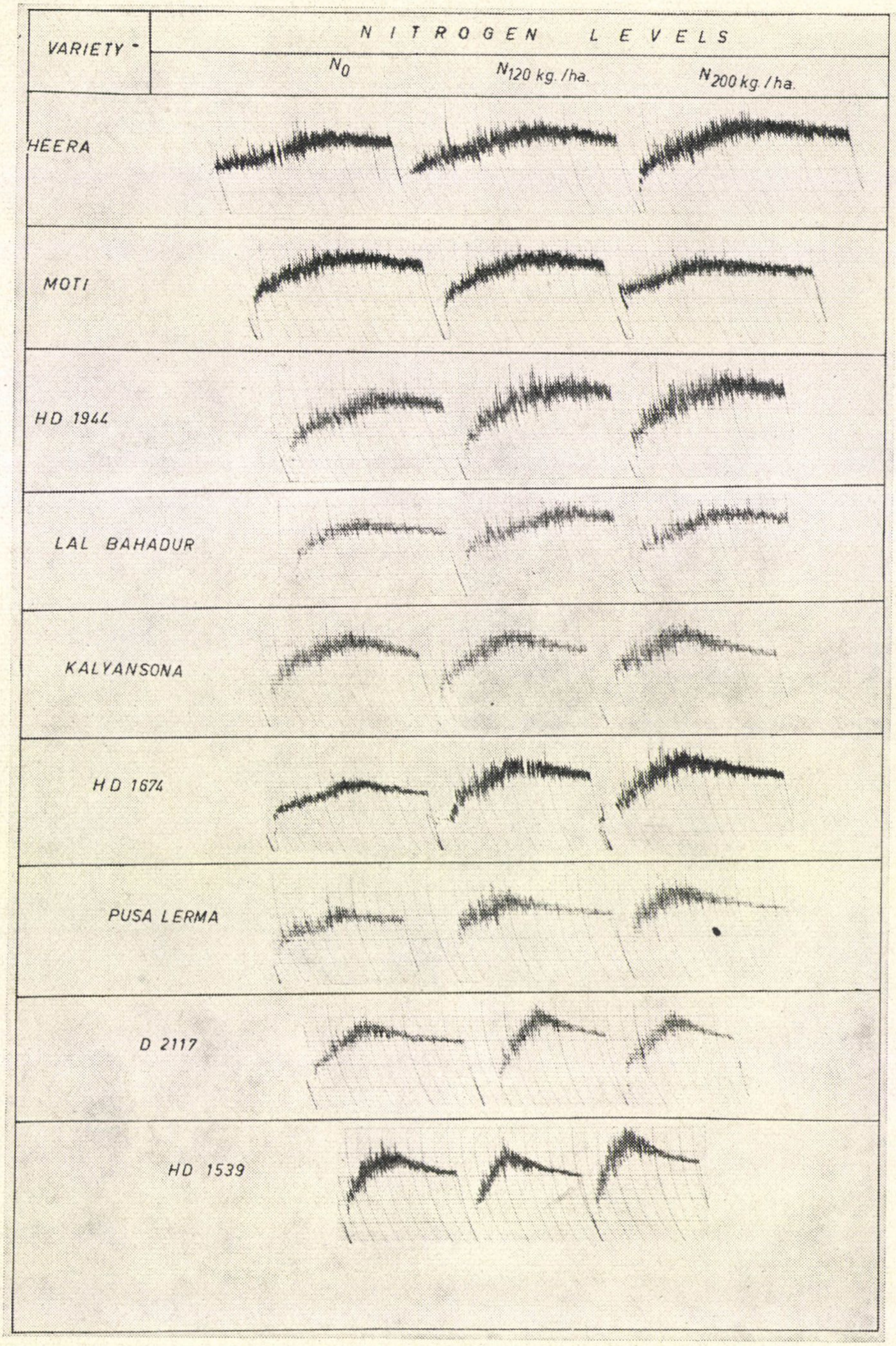

Fig. 2. Effect of nitrogen fertilization on dough behaviour. Mixogram characteristics of improved wheat varieties

Table 4

Correlations between protein percentage and dough characters

Levels	P×MT	P×S	P×PH	P×E	P×AN	P×AR
N_0	0.113	−0.257	0.158	0.458	−0.022	0.112
	(0.30)	(0.70)	(0.42)	(1.37)	(0.06)	(0.30)
N_1	−0.484	−0.401	−0.277	−0.128	0.388	−0.384
	(1.46)	(1.16)	(0.76)	(0.34)	(1.11)	(1.10)
N_2	−0.355	−0.455	0.242	0.278	−0.397	−0.424
	(1.00)	(1.35)	(0.66)	(0.76)	(1.14)	(1.24)

P — Protein; MT — Mixing time; S — Stability; PH — Peak Height; E — Elasticity; AN — Angle of Dough Development; AR — Area of Mixogram.

The figures within brackets show the *t*-values. Tabled value of *t* at 5% level of probability and 7 degrees of freedom is 2.365.

Effect of nitrogen: As for the effect of nitrogen, a significant increase in elasticity was found with 120 kg.N/ha but with additional dose there was no effect. The same trend was observed for peak-height and protein (Table 3 and Fig. 2).

Correlation coefficients between protein percentage and each of the other 6 characters for each level of fertilization were worked out and the data presented in Table 4. None of the coefficients were found to be statistically significant which means that dough characters are independent of protein content.

Acknowledgement

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Table 5

Varieties in order of merit for the various characters

Dough development time (in min.)	Heera 4.66	HD1944 3.50	HD1674 3.33	EA222-1 3.25	HD1949 3.03	Kalyansona 2.50	Pusa Lerma 2^{6}50	D2177 2.50	HD1539 1.83
Stability (in min.)	HD1944 2.08	Heera 2.00	EA222-1 1.83	HD1949 1.75	Kalyansona 1.58	HD1674 1.58	Pusa Lerma 1.08	HD1539 0.83	D2117 0.59
Area (cm^2)	Heera 9.07	HD1944 7.77	Kalyansona 7.20	HD1949 6.40	HD1674 5.50	Pusa Lerma 5.37	EA222-1 5.17	D2117 5.03	HD153 4.50
Elasticity (in mm)	HD1944 14.00	D2117 11.33	HD1539 11.17	Heera 11.00	Kalyansona 10.17	Pusa Lerma 10.00	HD1949 9.33	HD1674 8.67	EA222-1 8.67
Angle of dough development (in degrees)	HD1539 75.00	D2117 66.00	Kalyansona 64.00	Pusa Lerma 59.67	HD1949 51.00	HD1944 49.67	EA222-1 49.00	HD1674 48.67	Heera 36.00
Peak height (in mm)	HD1539 44.33	Kalyansona 44.00	HD1944 43.33	D2117 42.66	HD1674 41.00	HD1949 39.33	Pusa Lerma 39.00	EA222-1 38.00	Heera 38.00
Protein (%)	Pusa Lerma 14.36	HD1539 13.44	D2117 13.25	Heera 12.99	HD1949 12.74	HD1674 12.64	HD1944 12.54	EA222-1 12.29	Kalyansona 11.93

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DISTRIBUTION OF TOTAL B, Cu, Mn AND Mo CONTENTS IN THE PROFILES OF SOME SOIL TYPES IN THE LITTLE PLAIN*, AND ITS RELATIONSHIP TO CERTAIN SOIL CHARACTERISTICS

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The total boron, copper, manganese and molybdenum contents were determined in profiles of humous alluvial, chernozem-meadow, typical meadow and muck-meadow soils developed above the detrital cone of the Danube as well as in those of reclaimed and plotted fens. In all the studied soil types the micro-elements accumulated in the upper layer, in quantities 1.67—3.06 times as high as in the parent or base rock. However, owing to a stratification of alluvial origin a micro-element accumulation was sometimes found in the B or C horizons too. The multiple regression equations show that the boron, copper and manganese contents were the highest in soil samples containing 8.5, 7.1 and 5.0 per cent organic matter respectively. In samples with either extremely low or extremely high organic matter contents the quantity of the micro-elements was equally low. The molybdenum content, on the other hand, increased in direct ratio to the increase in the organic matter contents of the soil samples. The copper content was higher in soils rich in stable humus, while the molybdenum content in those rich in non-stable humus.

In soils of low organic matter content the biological and adsorptive accumulation of the microelements studied were equally significant, while in soils with high organic matter contents the molybdenum content accumulated mainly biologically, and the copper and manganese contents through adsorption. The studied microelement contents were not influenced by solubility conditions dependent on chemical reaction (pH).

Introduction

Soils in the Little Plain are mostly of an alluvial, chernozem, meadow or moory character. They are especially suitable for studying the vertical distribution of microelement content, as the different types of soils are directly side by side, and their organic matter contents range between wide limits.

Of the most important five microelements the distribution of the total zinc content in these soils was studied by Six—Lukácsy (1969), therefore this time only the total contents of boron, copper, manganese and molybdenum were investigated.

The vertical distribution of the total microelement contents depends on many factors. One of these factors is the type of the soil.

According to investigations made by Pejve (1964), Bajescu—Chiriac (1964) and Palaveev (1958) the distribution of the total boron content in

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* Region in the north-western part of Hungary.

chernozem soils is rather uniform. GYŐRI (1958) found boron accumulation in the lower horizons of a moor profile.

According to DONCHEV (1959), ABABI *et al.* (1965), BAJESCU—CHIRIAC (1964), GYŐRI (1958, 1962), DOBROLYUBSKIY—KOZULYA (1966) and HOHLOVA (1967) the total copper content in chernozem soils generally decreased with depth; GYŐRI (1958) found the same phenomenon in a meadow soil. The distribution of the copper content in the profile was found to vary in moors by WINOGRADOW (1954), GYŐRI (1958) and DONCHEV (1959), while in Punjab soils by RANDHAWA—KANWAR (1964).

In the chernozem soil profiles studied by VLASYUK (1964), WINOGRADOW (1954), DOBRITZKAYA (1967), HOHLOVA (1967), ANDONIKOV *et al.* (1967), REID—WEBSTER (1969), BAJESCU—CHIRIAC (1964) and GYŐRI (1962) the total manganese content was the highest in the upper horizon, however, in other chernozem soils a different distribution was found by ABABI *et al.* (1965), SZÜCS—ELEK (1962), CZOPF (1964), GYŐRI (1958), and TONKONOZHENKO (1964). In meadow and moor soils, on the other hand, it was in the lower horizons that GYŐRI (1958), REID—WEBSTER (1969) and CZOPF (1964) observed a manganese accumulation.

According to results obtained by GORBACHEVA (1971), DOBRITZKAYA (1967), ABABI *et al.* (1965), and GYŐRI (1962) the total molybdenum content also accumulates in the upper layers of chernozem soils; a different distribution of molybdenum was found, however, by HOHLOVA (1967), WINOGRADOW (1954), DOBRITZKAYA (1962, 1964), ADERIHIN—PROTASOVA (1970), ANDRONIKOV *et al.* (1967), TONKONOZHENKO (1964), SZÜCS—ELEK (1962) and CZOPF (1964). A varying molybdenum distribution was found by CZOPF (1964) in meadow and alluvial soils too. In moor soils studied by VISINTINI ROMANIN (1961), GYŐRI (1958) and SZALAY *et al.* (1970) the molybdenum accumulated in the horizon immediately below the surface soil.

The literary data listed give evidence of the fact that the distribution of microelements in the soil profile depends — in addition to the type of the soil — on other factors too.

PENKOV (1967), BAJESCU—CHIRIAC (1968, 1964), VINAYAK *et al.* (1967), SZÜCS—ELEK (1962), DOBRITZKAYA (1964), KERESZTÉNY—NAGY (1960) and VISINTINI ROMANIN (1961) pointed out a positive correlation between total microelement content and organic matter content, though GORLACH (1963) found no such correlation in the alluvial soils studied by him.

An unambiguous positive correlation was similarly demonstrated between clay fraction or permeability and total microelement content by OBUHOV (1968a), LUPINOVICH—DUBIKOVSKIY (1966), RANDHAWA—KANWAR (1964), CONNOR *et al.* (1957), VERIGINA (1961) and GYŐRI (1962) in relation to copper, and by GONZALES GARCIA—MAZUELOS VELA (1960b) and VLASYUK (1964) as to manganese.

The relationship between microelement contents and pH-value was studied by BAJESCU—CHIRIAC (1964) and PENKOV (1967) in relation to boron, by VINAYAK *et al.* (1967), VLASYUK (1964) and SZÜCS—ELEK (1962) as to manganese, and by SZÜCS—ELEK (1962) again concerning the molybdenum content. Correlations were either dependent on the site and soil type, or no correlation at all could be pointed out. In some soil samples FILIPOVIC *et al.* (1961) found a negative correlation between the total copper content and the pH-value.

Lime content showed a positive correlation with the total boron content and a negative one with the copper content in samples studied by OBUHOV (1968b), and a negative correlation again with the manganese content according to GONZALES GARCIA—MAZUELOS VELA (1960a).

It was with the view of a detailed further study on these correlations that soil profiles of the plain in north-western Hungary were examined.

Material and Method

The soil profiles studied were obtained from the districts of Mosonmagyaróvár, Horvátkimle and Lébény. Their parent rock is an alluvial deposit of the Danube. Their types were determined by the methods of STEFANOVITS (1963) and SZABOLCS *et al.* (1966).

The data of the basic examination are found in a publication by KERESZTÉNY (1970). The total microelement contents in them were determined from solutions obtained by soil digestion performed in a way described by RINKIS (1960). The boron content was determined with the method described by KERESZTÉNY (1966): with quinalizarin reagent after a solution containing chloride too was added; the copper content with dithizone in the presence of ascorbic acid, according to the description of KERESZTÉNY (1970); the manganese content with ammonium-peroxy-disulphate by the use of a catalyzer mixture described by SZÉKELY (1963); and the molybdenum content was determined with the technique used by KERESZTÉNY (1968). The examination data are all average values of the determinations made from two different digestions, this made the computation of the significant differences possible.

The distribution of the average microelement contents in the profiles of the studied soil types was determined in the following way: data of the same horizons within a soil profile were averaged separately, then, taking the values of identical horizons in profiles belonging to the same soil type for replications, the mean values were calculated for each horizon. In this way significant differences could be determined between the microelement contents of the horizons. When averaging we did not use the data of two moor soil profiles (Nos 358. and 368.) as samples were not taken from each horizon separately; however, in the correlation calculations these profiles were used as well.

Taking all samples as a basic mass multiple regression equations were set up to express the numerical correlations between the microelement contents and other soil research data. The calculations were made according to SNEDECOR (1957).

Results

The total microelement contents in profiles of different soil types are presented in Tables 1—5.

According to the data of Table 1 the microelement contents studied decreased with depth in all three humous alluvial soil profiles; in the 120—

Table 1

Total microelement contents in humous alluvial soils

Number	Depth of sampling cm	Total microelement content mg/kg			
		B	Cu	Mn	Mo
114	0—20	15.0	23.0	499	1.04
	20—40	14.2	21.2	492	0.97
	40—80	11.0	19.0	284	0.72
	80—100	12.8	19.9	469	1.41
	100—120	9.2	16.4	335	0.85
	120—150	14.4	25.8	476	1.05
	S.d.$_{5\%}$	1.6	4.8	99	0.38
236	0—20	18.2	21.5	435	1.06
	20—40	14.6	20.0	403	1.03
	40—60	14.2	17.1	391	1.00
	S.d.$_{5\%}$	4.6	11.2	0	0.15
247	0—30	15.8	20.9	246	1.13
	30—60	13.0	21.7	185	0.75
	60—90	15.8	21.2	178	0.57
	90—100	15.2	17.5	183	0.61
	100—200	11.4	16.6	164	0.49
	S.d.$_{5\%}$	3.4	6.8	11	0.07

150 cm layer of profile No. 114., however, boron and copper, and in the 80—100 cm layer manganese and molybdenum showed significantly high values.

While in the profiles of chernozem-meadow soils contained in Table 2 the microelement contents similarly decrease with depth, in the lower horizons of each profile there occur significantly high values, especially in the manganese and molybdenum contents.

The data of Table 3 show that in the profiles of the typical meadow soils studied the distribution of the microelements is similar to that in chernozem meadow soils. It is interesting that in profile No. 109. the total boron and manganese contents show unusually high values in the 100—120 cm layer, while the total copper content in the 80—100 cm layer.

The same can be said about the vertical distribution of the total boron, copper, manganese and molybdenum contents in the muck-meadow soils presented in Table 4 as in the case of typical meadow soils.

Table 5 shows that in the studied reclaimed and plotted fens the vertical distribution of the four microelements was the same as in the former soil types.

Table 2

Total microelement content in chernozem-meadow soils

Number	Depth of sampling cm	Total microelement content mg/kg			
		B	Cu	Mn	Mo
74	0—20	16.2	29.7	525	1.48
	20—40	21.6	30.7	537	0.96
	40—60	16.6	31.7	394	1.08
	60—100	17.0	34.0	396	0.96
	100—140	13.6	12.9	423	0.86
	$S.d._{5\%}$	2.6	5.2	68	0.27
93	0—20	20.0	37.2	504	1.25
	20—33	14.4	33.4	441	1.22
	33—52	13.2	29.7	412	1.27
	52—63	15.6	37.8	311	1.04
	63—90	11.2	30.5	594	1.18
	90—105	13.2	22.9	535	1.05
	105—125	12.8	18.1	693	1.26
	125—145	14.2	18.5	520	1.13
	145—170	3.8	9.4	224	0.52
	$S.d._{5\%}$	2.8	6.5	77	0.22
102	0—20	18.0	61.7	345	1.12
	20—30	19.0	36.2	386	1.05
	30—62	16.4	34.5	389	1.02
	62—75	15.0	39.4	246	0.80
	75—120	13.4	30.3	409	0.65
	120—140	11.6	29.2	363	0.61
	$S.d._{5\%}$	4.4	7.3	16	0.12
175	0—20	15.8	33.9	466	0.89
	20—40	16.4	42.1	434	0.95
	40—60	16.0	43.0	289	1.08
	60—100	11.2	28.9	247	2.27
	100—120	8.0	13.6	90	0.93
	120—150	7.0	9.3	56	0.39
	$S.d._{5\%}$	2.8	5.6	76	0.51
240	0—30	21.6	31.6	546	1.20
	30—50	20.4	30.3	740	1.09
	50—70	20.0	28.8	780	1.08
	70—90	20.4	31.0	635	0.99
	90—100	18.6	35.6	437	0.92
	100—120	18.6	51.0	317	0.75
	120—130	17.0	26.4	387	1.25
	$S.d._{5\%}$	3.8	3.6	74	0.10

Table 3

Total microelement content in typical meadow soil profiles

Number	Depth of sampling cm	Total microelement content mg/kg			
		B	Cu	Mn	Mo
109	0—20	14.0	50.3	262	0.95
	20—50	12.6	42.0	208	0.96
	50—80	11.6	37.0	193	1.20
	80—100	9.8	50.8	191	1.23
	100—120	13.4	26.4	249	1.21
	S.d.$_{5\%}$	2.0	6.3	57	0.22
166	0—20	17.2	48.1	271	0.78
	20—60	13.0	34.6	247	0.58
	60—70	8.2	27.5	167	0.62
	70—90	6.0	8.0	58	0.20
	S.d.$_{5\%}$	3.6	3.6	22	0.34

Table 4

Total microelement content in muck-meadow soils

Number	Depth of sampling cm	Total microelement content mg/kg			
		B	Cu	Mn	Mo
404	0—20	19.6	64.1	428	1.43
	20—50	15.0	45.9	384	1.92
	50—70	13.8	26.0	232	1.53
	70—80	15.0	12.1	421	2.15
	80—100	17.8	14.7	371	1.54
	100—120	14.6	15.4	194	1.68
	S.d.$_{5\%}$	2.6	11.2	68	0.70
410	0—5	16.8	61.4	358	1.38
	5—30	19.6	61.0	388	2.16
	30—40	13.8	24.5	737	2.50
	40—50	15.0	11.9	748	2.30
	50—70	13.8	22.0	646	1.65
	70—90	14.4	12.4	458	1.58
	S.d.$_{5\%}$	3.8	6.6	85	0.56

Table 5

Total microelement content in reclaimed and plotted fens

Number	Depth of sampling cm	Total microelement content mg/kg			
		B	Cu	Mn	Mo
163	0—20	14.8	51.1	449	7.20
	20—40	13.2	20.0	185	4.42
	40—60	12.8	12.9	208	1.40
	S.d.$_{5\%}$	0.0	6.1	30	2.90
189	0—10	14.4	63.9	548	4.74
	10—20	18.2	67.8	225	15.72
	20—40	14.2	73.6	149	5.02
	40—50	14.0	15.8	190	1.75
	50—70	12.2	11.2	118	0.92
	70—90	7.8	7.9	89	0.55
	90—110	12.0	17.1	139	0.93
	S.d.$_{5\%}$	3.8	5.6	32	2.52
197	0—20	14.6	57.0	181	7.34
	20—30	13.6	14.9	173	5.42
	30—50	10.6	12.2	144	4.18
	50—70	11.6	13.2	161	1.01
	70—90	17.4	12.7	148	0.57
	S.d.$_{5\%}$	6.8	2.0	14	0.37
358	0—40	15.6	49.0	231	15.96
	40—160	9.4	38.3	156	19.71
	160—180	13.0	41.2	197	16.03
	S.d.$_{5\%}$	4.0	27.0	30	3.80
368	0—130	13.8	50.7	334	9.16
	130—170	16.6	33.2	157	6.88
	170—250	11.4	22.6	175	8.76
	S.d.$_{5\%}$	8.4	10.0	30	5.10

The extremely high microelement contents occurring in the lower horizons of certain profiles in all five soil types are probably caused by the fact that the parent rock of the soils studied is an alluvial deposit of the Danube which is characterized by stratification.

The average microelement contents in the profiles of the studied soil types are shown in Table 6.

As seen in Table 6 the total microelement content decreases with the depth in all five soil types. Only the muck-meadow soils are exceptions as

Table 6

Average total microelement contents in the profiles of the soil types studied

Soil types	Horizon	Depth of horizon cm	Total microelement content mg/kg			
			B	Cu	Mn	Mo
Humous alluvium	A	0—24	15.8	22	393	1.08
	B	24—46	14.8	21	360	0.92
	C	46—110	12.4	18	320	0.86
	S.d.$_{5\%}$		3.0	2	62	0.35
Chernozem meadow soils	A	0—22	18.4	39	480	1.20
	B	22—36	18.4	35	470	1.08
	BC	36—78	16.4	36	388	1.04
	A_b^*	78—100	14.2	35	394	1.20
	C	100—158	11.8	19	322	0.86
	S.d.$_{5\%}$		2.4	11	134	0.47
Typical meadow soils	A	0—20	15.6	41	267	0.9
	B	20—56	12.8	31	230	0.8
	BC	56—84	9.6	28	180	0.6
	C	84—106	9.8	9	160	0.4
	S.d$_{5\%}$		10.2	17	230	0.5
Muck-meadow soils	A	0—12	18.2	65	393	1.4
	B	12—40	17.6	54	385	2.1
	BC	40—56	13.8	26	485	2.0
	C	56—104	15.0	15	480	1.9
	S.d.$_{5\%}$		7.2	18	480	1.0
Reclaimed and plotted fens	A	0—26	15.0	59	312	7.3
	B	26—40	13.6	17	183	3.7
	C	40—86	12.2	13	160	1.3
	S.d.$_{5\%}$		2.4	13	160	3.0

* buried ancient horizon

regards their manganese and molybdenum contents; the differences between the horizons are not, however, significant. The total copper content in the A-horizons increases considerably at higher organic matter contents. The A- and B-horizons of reclaimed and plotted fens are characterized by a high molybdenum content.

In order to find out the extent of microelement accumulation we compared the microelement contents in the upper layers of the profiles to

Table 7

Mean values of microelement proportions determined in the uppermost and lowermost horizons of the soil profiles studied, and confidence limits of mean values at the P = 0.05 level

B	Cu	Mn	Mo
1.67±0.57	3.06±0.80	2.26±1.00	2.87±1.57

those in the lowermost layers, that is, in the parent rock. The results thus obtained are shown in Table 7.

According to the data of Table 7 the accumulation of all the studied microelements as compared to the parent rock is statistically proved on the average of all the soil profiles. There is a 150 per cent accumulation of boron and about 300 per cent of copper. The difference between the two is significant. The great deviation in the data of the 15 profiles (CV% = 13.3—27.9) is partly explained by the fact that owing to a stratification in the soils of alluvial origin the C-horizon is not the parent rock, only a basic rock. The great deviation of the ratios relative to the molybdenum content is caused, on the other hand, by the fact that in this respect significant differences can be found between the soil types studied (F [Fisher] = 4.18, which is significant at P = 0.05 level). The Dixontest proves at a P = 0.02 level that the 770 per cent accumulation of the molybdenum content in the upper layer of a reclaimed and plotted fen is an extremely high value compared to a 90—240 per cent accumulation in the other soil types studied.

When studying the factors of the vertical distribution of microelements we obtained the following multiple regression equations:

$$B = -0.4492\,\mathrm{Sz} + 26216\,(\mathrm{Sz})^{0.5} - 0.9752\,\mathrm{hy} + 6.6308\,(\mathrm{hy})^{0.5} - 0.2870\,\mathrm{pH} +$$

$$+0.1482\,\mathrm{Ca} - \frac{1.178}{\mathrm{Ca}+2} + 0.1624\,\lg Q + 4.06$$

$$\mathrm{Cu} = -1.854\,\mathrm{Sz} + 9.902\,(\mathrm{Sz})^{0.5} + 1.931\,\mathrm{hy} + 9.512\,(\mathrm{hy})^{0.5} + 0.006\,\mathrm{pH} +$$

$$+0.1936\,\mathrm{Ca} + \frac{11.89}{\mathrm{Ca}+2} + 9.370\,\lg Q - 16.00$$

$$\mathrm{Mn} = -26.59\,\mathrm{Sz} + 119.41\,(\mathrm{Sz})^{0.5} + 31.56\,\mathrm{hy} + 51.10\,(\mathrm{hy})^{0.5} +$$

$$+6.98\,\mathrm{pH} + 10.707\,\mathrm{Ca} + \frac{136.2}{\mathrm{Ca}+2} - 50.24\,\lg Q - 84.72$$

$$\mathrm{Mo} = 0.2661\,\mathrm{Sz} + 0.1529\,(\mathrm{Sz})^{0.5} - 0.2297\,\mathrm{hy} + 1.4895\,(\mathrm{hy})^{0.5} +$$

$$+ 0.1229\,\mathrm{pH} + 0.01503\,\mathrm{Ca} + \frac{2.276}{\mathrm{Ca}+2} - 0.8524\,\lg \mathrm{Q} - 1.29$$

In the above equations "B", "Cu", "Mn" and "Mo" mean the mg/kg microelement contents (total microelement), "Sz" means the percentage organic matter content, "hy" the hygroscopic value as determined by Sik's method, "pH" the value of chemical reaction measured in an aqueous suspension, "Ca" the percentage lime content, and "Q" means the humus stability index determined by Hargitai (1955). The latter is the quotient of extinction values measured at 533 nm in soil samples extracted with 1 per cent NaF and 0.5 per cent NaOH respectively.

Table 8

t-(Student) and P-(probability level) values characterizing the reliability of regression coefficients in multiple regression equations set up for total microelement contents; determination coefficients characterizing the extent of multiple correlation (R^2)

Variables	B		Cu		Mn		Mo	
	t	P	t	P	t	P	t	P
Sz	3.03	0.01	3.00	0.01	4.25	0.001	1.94	0.10
$(\mathrm{Sz})^{0,5}$	2.58	0.05	2.35	0.05	2.02	0.05	0.16	—
hy	1.62	0.20	0.76	0.50	0.89	0.40	0.41	—
$(\mathrm{hy})^{0,5}$	3.03	0.01	1.04	0.40	0.41	—	0.74	0.50
pH	0.57	—	0.00	—	0.24	—	0.26	—
Ca	4.86	0.001	1.52	0.20	6.00	0.001	0.53	—
$\frac{1}{\mathrm{Ca}+2}$	0.55	—	1.22	0.40	1.00	0.40	1.05	0.40
lg Q	0.29	—	4.01	0.001	1.55	0.20	1.66	0.10
R^2	0.57		0.70		0.60		0.73	

The reliability of the regression coefficients as well as the determination coefficients belonging to the equations are shown by the data of Table 8.

According to the data of Table 8 in the soil profiles studied the total boron, copper and manganese contents showed a significant curvilinear, while the total molybdenum contents a nearly significant linear correlation with the organic matter content. Regarding the other independent variables to be average size, the total microelement contents were only plotted against the organic matter content. This is illustrated in Fig. 1.

Fig. 1 shows that the total boron, copper and manganese contents of the studied soils increased with the increase of the organic matter content, then — having reached a maximum — decreased. The highest boron content was found at 8.5 per cent, the highest copper content at 7.1 per cent while the highest manganese content at 5.0 per cent organic matter content. This extremely curvilinear correlation cannot be caused by qualitative differences between the humus contents of soils with higher and lower organic matter contents, since in the multiple regression equations the Q-value, which expresses the humus quality, is among the independent variables. More probably in organic matters at an initial stage of decomposition the concentration of biologically accumulated microelements is lower than in those at an advanced stage of humification.

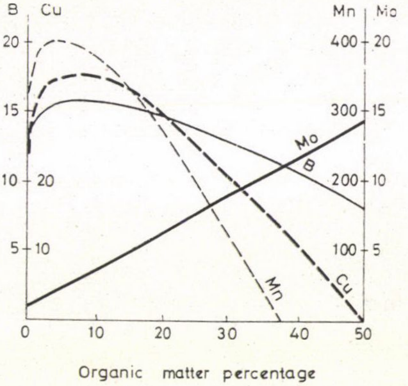

Fig. 1. Changes in the total microelement contents of soil samples studied as a function of their organic matter contents (mg/kg)

A similar correlation was observed in relation to the total zinc content by Six (1968, 1970, 1971a, 1971b) and Six—Lukácsy (1969, 1970, 1971). Namely, in mineral soils a positive correlation was found between the total zinc content and the organic matter content; in moor soils, on the other hand, the maximum zinc content appeared at a 18.75 per cent organic matter content, which is a considerably higher value than that characteristic of the highest boron, copper or manganese contents.

Fig. 1 shows that at a 40 per cent organic matter content the total manganese content ought to have a negative value, which is a sheer impossibility. This apparent fault is caused by the fact that the curves refer to conditions when the other soil characteristics correspond to the average values of the samples considered. When, however, the organic matter content of a sample is 40 per cent, then for example it is improbable that its hy-value only corresponds to the mean value — 2.83 — of the soil samples examined. Negative values possibly appearing at the descending branch of the curve thus mean no error of calculation or evaluation; they refer to the cases not occurring in practice.

Table 8 and Fig. 1 show that the total molybdenum content — unlike the other three microelement contents — increases in direct ratio to the organic matter content. This correlation is another confirmation of the statement of Keresztény—Nagy (1960), namely, that the ratio between the organic matter content and the organic matter bound molybdenum content is approximately constant in the soil samples.

According to the data of Table 8 the correlation between the microelement contents and the hy (hygroscopic) value is very loose; it is significant and curvilinear only as regards the boron content.

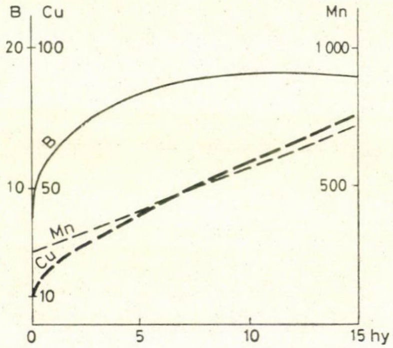

Fig. 2. Changes in the total microelement contents of soil samples studied as a function of their hy-values (mg/kg)

Fig. 2 clearly shows that in the soil samples examined there is a linear or almost linear correlation between the increase of the copper and manganese contents and the hy-value, while the boron content reaches a maximum at the 11.5 hy-value. The correlation for the molybdenum content is similar to that of the boron content; its probability level, however, does not exceed $P = 0.50$ so it is not shown in the figure. The hy-value is primarily in correlation with the specific surface of the soil particles, but to a lower extent also with the organic matter content. The equations and Figs 1 and 2 show that in soils with low organic matter contents the accumulation of the microelements studied takes place in both biological and adsorptive ways, but in those with high organic matter contents molybdenum accumulates biologically while copper and manganese through adsorption.

It was surprising that — as seen in Table 8 — no correlation was found between the microelement content and the pH-value in the examined soil samples. This was partly due to the fact that their pH-values — with the exception of two samples — ranged between very narrow limits (6.9—8.9).

A similar phenomenon was observed, however, by Six (1968, 1970, 1971a, 1971b) and Six—Lukácsy (1969, 1970, 1971) in relation to zinc.

As to boron and manganese contents, highly significant correlation was found between the microelement contents and the lime contents of the soil samples. The correlations are presented in Fig. 3.

According to the evidence of Fig. 3 the correlations between the microelement contents and the $CaCO_3$ content are approximately linear, if the initial sections of the curves corresponding to less than 4—5 per cent lime content are not taken into consideration. Thus, chemisorption occurring in the lime grains increased the accumulation of copper and boron; it increased the manganese content to a very great degree, while it decreased the molybdenum content.

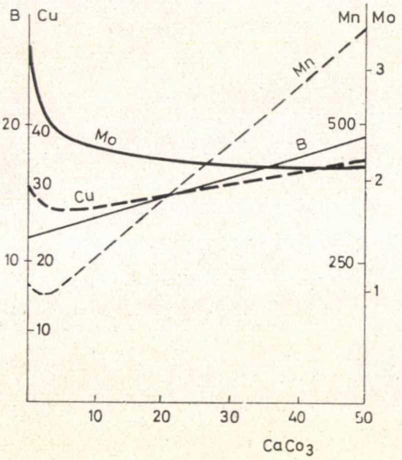

Fig. 3. Changes in the total microelement contents of soil samples studied as a function of their $CaCO_3$ contents (mg/kg)

This correlation is confirmed by the results of Tölgyesi *et al.* (1970). Namely, in the acidic sandy soil studied by them the total content of copper and manganese was much lower while the molybdenum content in the surface layer somewhat higher than in calciferous sandy soils.

According to the above equations and Table 8 the total copper content showed a highly significant positive, while the molybdenum content at the $P = 0.10$ level a significant negative correlation with the logarithm of the humus stability number (Q). Thus, the accumulation of copper is promoted by good quality, stable humus, while that of molybdenum by non-stable humus.

According to the data of the last line in Table 8 the dispersion of the microelement contents in the samples studied can be explained to 57—73 per cent by soil characteristics included in the equations as independent variables.

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ANATOMICAL STUDIES ON ROOT GALL OF CHICORY (CICHORIUM INTYBUS L.)

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Gall formation in chicory roots induced by the infection of root knot nematodes, started with the proliferation of parenchymatous tissues in the centre resulting in hypertrophied growth. Due to the increased number and size of the parenchymatous cells in the cortex, some of the tissues died due to excessive maceration and were added to the periderm. The periderm started encircling some of the living parenchymatous cells of the cortex, thus resulting into the formation of large cavities. The development of tracheids commenced from the transverse series of initials which later on were distributed all through, and at certain places the spiral tracheids enclosed the living parenchymatous cells, thus forming large cavities. The spiral, circular, erect, straight, stratified, and isolated lobed arrangement of vascular elements was the main feature. Some of these tracheids underwent segmentation due to excessive proliferation of the surrounding parenchymatous cells, thus fragments got distributed throughout the gall. Identification of the nematode associated with gall formation in chicory roots is in progress.

Introduction

Galls on angiospermic plant parts are produced, in general on account of incidental disturbance caused by different types of organisms, resulting into abnormal exaggerated overgrowths in the form of enlarged swellings (VIGLIERCHIO 1971). Many of the allied aspects like morphogenic (KÜSTER 1925, BLUM 1963), histogenic (BIRD 1962, RAO 1965) and biochemical transformations (BALASUBRAMANIAN—RANGDSWAMI 1962, SETTY—WHEELER 1968) have been used as a tool for finding out the basic principles associated with the process of gall formation. The present study entails a comprehensive account of the anatomical aspects of galls on chicory (*Cichorium intybus* L.) roots to evaluate the steps required during the inception and formation of deformed cells and tissues.

Material and Method

In the commercial planting of chicory to be used for coffee blending during 1969—70, 1970—71, a greater proportion (15—20 per cent) of root knot nematode induced gall-formed roots were observed in the experimental area of Punjab Agricultural University, Ludhiana. Roots both normal and gall formed were collected at different stages of development and fixed in formalin–acetic acid–alcohol (FAA). The transverse sections of normal root and galls were cut and stained in safranin-fast green combination after the method of **Johansen** (1940) using xylene as a clearing agent. The photomicrographs were taken with a 35 mm camera attached to the microscope with an adapter.

Results

Plant symptoms of galled-plants comprised stunted growth, deficiency of nutrient elements, smaller chlorotic leaves and sterile seeds.

The infection started during the vegetative phase on the primary root, thus producing galls of protoplasmic type (**Küster**'s classification 1911, 1925) which were in the form of lobed, smooth, sessile, yellowish green to brownish, solid swelling (5—8 cm diameter) on the lateral side of the basal part of thicker conical tap root (Fig. 1).

Fig. 1. Chicory roots showing different stages of gall formation

A cross-section of a young healthy root had an unilayered epidermis (epiblem) without cuticle followed by a cortex consisting of 7—8 layers of large oval parenchymatous cells. The innermost cells of the cortex had many intercellular spaces, but the outer layers were compact. The articulated anastomosing laticifers were located in the cortex and mainly in the young, secondary phloem. The vascular tissue delimited by unilayered pericycle and endodermis was composed of a diarch primary xylem having annular, helical, scalariform and pitted tracheids and forminate vessels with bordered pits on their lateral walls.

Mostly roots having galls depicted secondary growth. The epidermis and most of the layers of the cortex had been replaced by a few layered periderm and persistent ray of parenchyma running through the secondary xylem from each protexylem pole. The secondary xylem formed on the inner face of the cambium consisted of vessel segments having oval and scalariform-reticulate type of pits on their lateral walls and secondary parenchymatous cells. These two kinds of cells were interspersed at first, but later the parenchyma was laid down in rays. The secondary phloem which occupied the greater area in the mature root was composed of sieve tubes, companion cells, laticifers and other parenchymatous cells. The "spoked" condition as reported by KNOBLOCH (1954) was seen in the phloem, probably because of the sharp distinction in the functions of the two types of cambial initials (Fig. 2).

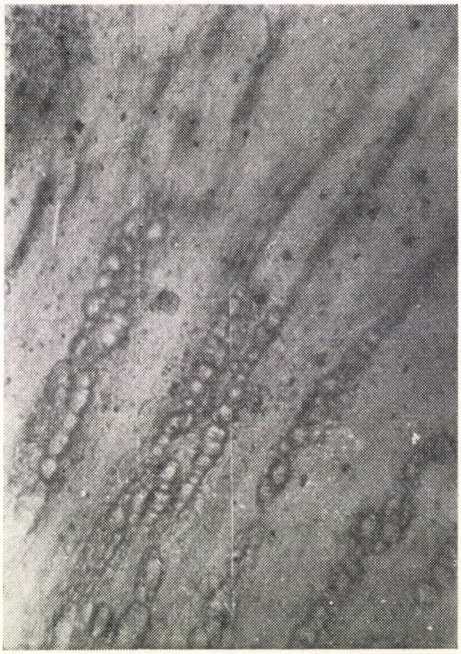

Fig. 2. Cross-section of normal chicory root showing spoked arrangement of phloem and normal tracheids. ×160

Fig. 3. Cross-section of chicory root gall showing early stages of development of cavity with dislocated tracheids. ×160

A cross-section of the gall did not show a clear differentiation of tissues. Significant anatomical changes were the cell wall dissolution, cellular hypertrophy and hyperplasia, abnormal xylem, and large periderm. The formation of the periderm was accelerated due to infection to occupy a larger area, encircling the living parenchymatous cells by extending towards the cortical regions. Possibly the stimulus causing the cellular hypertrophy and hyperplasia and later the maceration into groups in the gall tissues is chemical, probably the growth hormones (BIRD 1962, SETTY—WHEELER 1968) or some other allied chemicals like amino acids (Sonders—Burkholder 1948, Anders 1958) or the activity of certain enzymes like celluloses (Krüsberg 1960, Morgan—McAllen 1962) secreted by the organism during infection. In the process of hypertrophy, some of the original parenchymatous cells were killed and obliterated leaving larger or smaller cavities (Fig. 3). All the tissues of the gall whether parenchymatous or lignified were derived by the activity of the pericycle cells.

Fig. 4. Cross-section of chicory root gall showing spiral arrangement of tracheids and development of cavity. ×400

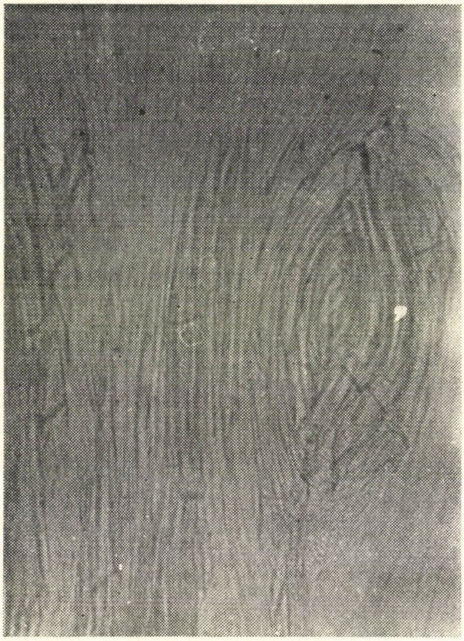

Fig. 5. Cross-section of chicory root gall showing stratified arrangement of tracheids. ×400

An interesting feature observed in the gall was an intricate vascularization consisting of lignified tracheids and vessels. The secondary parenchyma was also converted into vascular elements by developing pitting and lignification of the walls (Jones 1947). This type of vascularization possibly developed due to the invariable stimulation of the xylem elements during the initial formation of the tumor strands which divided repeatedly accompanied by a slight enlargement of the affected cells (Braun 1941). The vascular system in the gall adopted some peculiar arrangements of tracheids and vessels which were completely different from the normal. The tracheids originated from the transverse series of initials had undergone a lot of changes after infection resulting into a radial and oblique development. This in turn broke the continuity of the vascular elements. The tracheidal elements which became spiral (Fig. 4), started enclosing the living parenchymatous cells thus appearing as cavity (Fig. 4) in the beginning and later turning into erect straight and stratified structures (Fig. 5) having isolated and lobed, fabrication (Fig. 6) at certain places. This spiral, circular, lobed and stratified arrangement of the vascular elements was found to be the characteristic feature of this gall. Such types of anatomical deformities in gall formed tissues have rarely been reported earlier. These tracheids underwent segmentation late in the development of the gall too (Fig. 7). Thus in completely developed galls, the vascular elements were found mostly in the form of fragments which gave the appearance of mashes. Some of these fragments accumulated in the cavities, thus lost all the organic connections with the rest of the plant tissues. The possible reason for the segmentation of the tracheidal elements may be due to the induced expansion, elongation and excessive proliferation of the surrounding parenchymatous tissues or the tracheidal tissues themselves, thus spreading the fragments throughout. The tracheidal elements may expand or elongate, with the result that the walls get ruptured and the bordered pits on the tracheidal walls become laterally expanded. The elongation of the tracheids resulted in

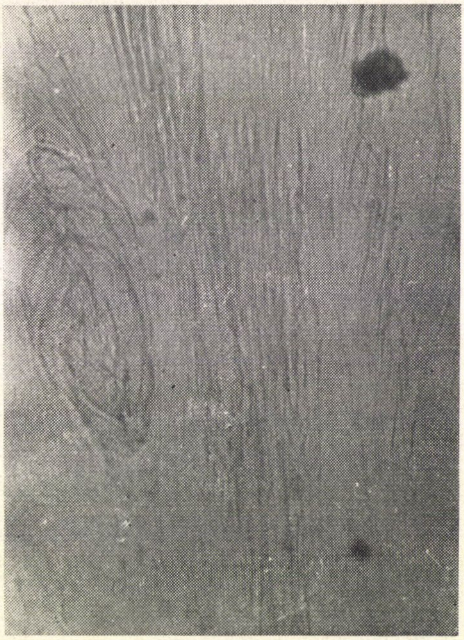

Fig. 6. Cross-section of chicory root gall showing isolated and lobed tracheids. ×400

Fig. 7. Cross-section of chicory root gall showing segmentation of tracheids. ×160

the separation of their spiral thickening, the disintegration of the lateral walls. The medullary rays also acquired some different arrangement. Both the uni- and the biseriate medullary rays expanded with overlapping margins. In the later stage of gall development, the rays became a completely discontinuous structure in a radial direction. This may block the lateral translocation between the central and peripheral tissues of the gall completely.

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THE INFLUENCE OF AGROTECHNICAL FACTORS ON THE EVAPOTRANSPIRATION OF RICE

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Two water depths, each with two N levels and two plant populations were compared during two rice growing seasons as to their influence on the ET of rice. ET was measured by embedding galvanised iron tanks in the rice field. An increase in the water depth caused significant increase in ET during the early period of rice growth. In the later period, there was no difference. ET was significantly different at the two levels of plant population at both water depths. The effect of N levels on ET was not consistent. However, the trend was that high N levels increased the ET at 5 cm water depth. The opposite was the effect at 20 cm water depth.

Introduction

The rate of water loss by the process of evapotranspiration (ET) is the result of five controlling factors, namely the climate, soil moisture, crop factors, soil properties and cultural practices. Of these five factors, the plant and soil factors are so closely interrelated to each other and also to the climatic factors, that very little is known about their individual roles in the overall ET processes. Though the agrotechnical factors are known to be equally important, their investigation has not yet received the same attention as other meteorological factors. The present work, therefore, aims at presenting the effect of agrotechnical factors on the ET of rice. This was accomplished by different levels of water depth, plant populations and nitrogen (N) levels.

The effect of different water management practices on ET has been reported by some workers in Japan (FUJIOKA 1960, TSUTSUI 1966, NAKAGAWA 1969). The above studies show that the ET from the rice field would be minimal at shallow water depths.

CHAUDHURY—MAHAPATRA (1963), and CHAUDHURY—PANDEY (1966) in India reported that ET increased when the rice crop was manured with ammonium sulphate. These experiments were in pots.

There are no published data to show the water use pattern of rice with respect to plant population. But, the available data from other crops show that the plant population within certain limits does not appreciably increase the total rate of water use (STERN 1965, LEMON 1966).

Material and Method

The study was conducted during the years 1968 and 1969 at the State Farm, Mezőtúr, a typical rice growing area of the Hungarian great plain. The elevation is 83 m and it is situated 47° north, 20.31° east. The soil is solonchak. The average annual precipitation is 550 mm.

There were 3 factors, namely, water depth (5 and 20 cm), N levels (40 and 80 kg/kh), and plant population (100 and 200/sq.m) with all their combinations. Thus there were 8 treatments replicated 4 times.

The equipments used in the studies were galvanised iron tanks with an area of 2500 sq. cm. The tanks were embedded in the centre of the rice field. ET was measured daily using the Héni—Tóth type gauge (Figs 1, 2, 3). The meteorological elements such as rainfall, temperature, relative humidity and evaporation were measured by the appropriate instruments recommended for the purpose.

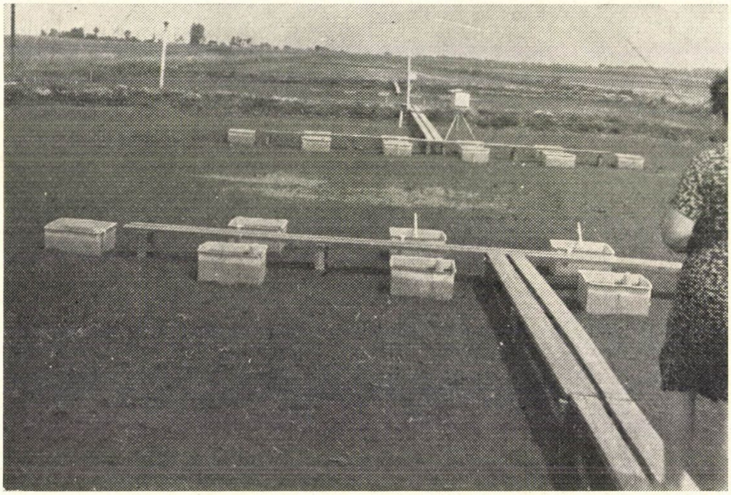

Fig. 1. A general view of the layout of the tanks in the rice field

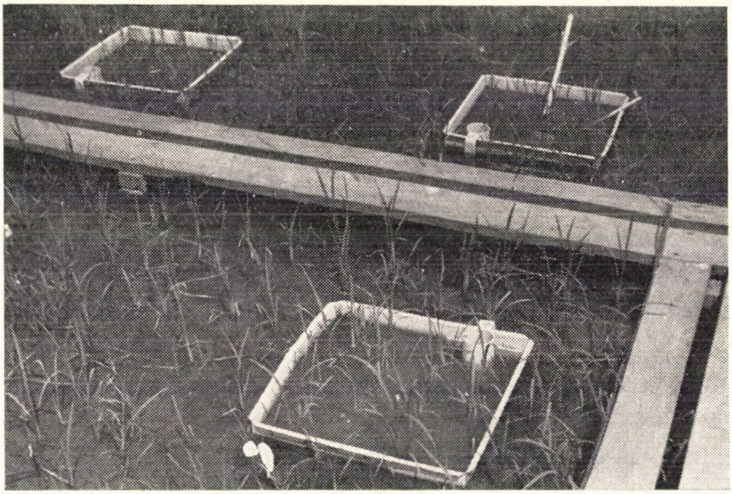

Fig. 2. Same one month after sowing

Fig. 3. Same at the active tillering stage

Results

Water depth and ET. The effect of water depth on ET during the growing period is presented in Table 1.

It was observed that ET increased with the water depth. During the year 1968, increase in ET at 20 cm as compared to 5 cm water depth was significant only during the early period (June). During 1969, however, ET at 20 cm water depth was significantly higher than at 5 cm, in June, July and August. In both the years, during the later period of growth, there was no significant difference in ET at the two water depths.

The higher ET observed at the 20 cm water depth compared to the 5 cm water depth may be due to the following reasons:

1. The differential water depth affects the energy components of solar radiation. Net radiation available for the latent heat of vaporisation is higher at 20 cm (VAMADEVAN 1970).

Table 1

Water depth and ET

Water depth	1968 ET (mms)				1969 ET (mms)				
	June	July	Aug.	Total	June	July	Aug.	Sept. (1–20)	Total
5 cm	159	185	118	462	143	168	161	89	561
20 cm	179	190	121	490	159	178	168	88	593
C.D. at 5%	8.0	N.S.	N.S.	8.0	1.9	1.3	1.1	N.S.	3.8

2. Plant height is seen to be affected by the water depth especially during the early period (VAMADEVAN 1970). It is greater in the 20 cm water depth than in the 5 cm. It is recognised that difference in plant height and the flexible nature of the crop could affect ET indirectly, by increasing the turbulence and aerodynamic roughness (MAKKINK 1962).

Table 2

Average air temperature recorded by the thermohygroraph 1969

Months	5 cm	20 cm
June	18.5	19.2
July	21.3	22.1
August	20.0	21.0
September	18.1	18.5

Table 3

The effect of fertilizers on the evapotranspiration of rice

N levels	5 cm ET (mm)					20 cm ET (mm)				
	June	July	Aug.	Sept.	Total	June	July	Aug.	Sept.	Total
40 kg/kh	152	179	116	—	447	182	190	126	—	498
1968 80 kg/kh	156	191	121	—	468	174	189	116	—	479
Difference	N.S.	N.S.	N.S.	—	N.S.	N.S.	N.S.	N.S.	—	N.S.
40 kg/kh	140	167	157	86	549	162	181	176	93	610
1969 80 kg/kh	147	171	166	92	575	157	177	161	84	578
C.D. at 5%	6.1	N.S.	4.7	4.7	8.6	1.3	N.S.	13.2	4.5	21.9

3. The conditions for receiving the sum rays are a little more favourable at the 20 cm water depth than at the 5 cm water depth (VAMADEVAN 1970).

4. Air temperature is a little higher above the rice population at the 20 cm water depth. The temperature recorded by the thermohygrograph is shown below (Table 2).

The effect of N levels on ET. The effect of N fertilizer at 40 kg/kh and 80 kg/kh levels in two different water depths is presented in Table 3. The Table shows that while N levels had no effect on ET during the year 1968, they significantly increased it during 1969. 80 kg/kh N increased the ET significantly during all the months except July at the 5 cm water depth. It had the opposite effect, however, at the 20 cm water depth. The effect of the N fertilizers on ET is not significant at either level, if the data are presented irrespective of the water depths, as shown in Table 4.

The effect of plant density on ET. The effect of plant density on ET during the two rice growing seasons is presented in Table 5. The Table shows that ET is not significantly affected by the two levels of plant densities at either water depth. Thus the data show that even if the density of the plant population is increased, ET will not increase much.

Table 4

The effect of N levels on ET. The values are averages over water depths

N levels	1968				1969				
	June	July	Aug.	Total	June	July	Aug.	Sept.	Total
40 kg/kh	167	185	121	473	151	172	167	90	580
80 kg/kh	165	190	118	474	152	174	164	88	578
Difference	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Table 5

The effect of plant density on the evapotranspiration of rice

Plant density	5 cm ET (mm)					20 cm ET (mm)				
	June	July	Aug.	Sept.	Total	June	July	Aug.	Sept.	Total
100 pl./sq.m	152	183	117	—	452	180	186	118	—	484
1968. 200 pl./sq.m	154	186	118	—	458	175	192	124	—	491
Difference	N.S.	N.S.	N.S.	—	N.S.	N.S.	N.S.	N.S.	—	N.S.
100 pl./sq.m	144	170	163	89	566	158	181	169	88	596
1969. 200 pl./sq.m	143	168	160	89	560	160	177	168	89	594
Difference	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

In the rice field, the total ET depends upon the energy available at the water surface and the rice population. As the field is flooded, maximum ET (potential evaporation) is always obtained. Under these conditions, plant population has little effect on the total ET. For example, the rice with 200 plants/sq.m. density, will intercept more energy than that with a lower population, resulting in higher transpiration. However, the evaporation from the water surface will be in proportion with the energy transmitted to the water surface. Thus evaporation from the lower population is higher than that from the higher population making up for the difference in the transpiration.

This study corroborates in a general way the findings of other workers in other field crops (STERN 1965). Thus meteorological factors tend to determine ET more than the plant densities.

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CORRELATIONS BETWEEN GRAIN YIELDS OF "A" STRAINS AND OTHER WHEAT CHARACTERISTICS

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Certain hybrid combinations have shown positive correlations between grain yield and plant height in the "A" strain as well as between the grain yield and the plant height of the mother plant. In such combinations selection by plant height may promote the isolation of highly productive plants. Correlation varies from combination to combination. Grain yields of strains have displayed no correlations with the length of the vegetative period, therefore, in the combinations examined selection for earliness does not involve any decrease in productivity under Hungarian conditions.

Introduction

Breeding for productivity would greatly be enhanced if characteristics related directly or indirectly with yield or the individual yield components were known. Investigations into correlations between yield and other wheat characteristics are, therefore, highly justified.

As ROBINSON (1951) pointed out, though breeders are interested in superior genotypes, they have to select individuals necessarily on the basis of the phenotype. Besides, most economic features — e.g. grain yield — are transmitted as a complex, in combination with other characters. When breeding for productivity characteristics correlated with yield may play the role of indicators. They may be characters of no importance by themselves; generally they are not even considered valuable. Inclusion of such indicator characters in the breeding programme would be reasonable only if selection for them meant a progress in breeding for the main characters (JOHNSON *et al.* 1955). According to KRONSTADT (1964) breeding for productivity is most promising when it takes each yield component separately and also their combinations into consideration. In this way breeders can make use of the biological correlations existing between the yield components. Intensive correlation studies were performed and data on correlations between several major economic features were published only in the last few years. CLARK—HOOKER (1926) pointed out a positive correlation between vegetative period and plant height on the one hand, and grain yield on the other. TORRIE (1936) found a positive correlation between plant height and grain yield. Positive correlation was found by BHIDE (1963) between number of shoots and grain weight per ear, grain weight per ear and ear length as well as between germination and stand density. Thousand-grain-weight had a strong positive effect on yield. Positive effect was exerted, further, by the density of stand and the number of days to flowering. Number of shoots, grain weight per ear and germination had but a minor influence on yield. Ear length showed a strong negative effect. LUKYANENKO (1966) studied the relationship between yield per unit area and other economic features of the plant and found a positive correlation between yield and leaf-rust resistance, grain yield and grain/straw ratio, total grain weight and average weight per ear; and a negative correlation between yield and time of earing plant height and grain/straw ratio. The author emphasizes the importance of evaluating the correlations correctly and points out that correlations found at Krasnodar are valid under those conditions only and show the wheat types to be preferred at that site. LEGRAND (1966) found positive correlations between yield and plant height, yield and earing time, and plant height and earing time respectively.

Literature thus suggests that correlation of a number of characteristics with yield in wheat depends to a great extent on the material examined and the conditions of the experimental site.

Material and Method

Studies were made in 1965, 1966 and 1967 at Martonvásár, in the culture plot of the wheat breeding group. Combinations were produced by making hybridisation among Hungarian, Soviet, Italian, French and German varieties. In the F_1, F_2 and F_3 generations plants were wide spaced (40×10 cm). In F_2 mass selection while in F_3 individual selection was carried out. In certain combinations selection was carried out on more than one occasion.

The selected mother plants were examined and the best ones were set in a lattice design experiment with four replications. The area of the plots was 0.5 m^2 each. In each plot 80 grains were sown with a spacing of 10×5 cm. Taking high germination percentage into consideration, this corresponded to half of the amount of grain used in practice.

Field observations were carried out in all four replications. Weather conditions were suitable to carry out the experiments. In case of insufficient rainfall irrigation was applied. The soil of the culture plot was rich in nutrients. Peas with helianthus were used as green crop preceding wheat.

Results

Correlations between grain yield and other characteristics of wheat were studied with a material examined in our "A" strain trials. Characteristics that could be readily observed, precisely measured and considered in selection as indicators were first studied.

Correlation between the yields of strains and the heights of plants was examined. Tables 1 and 2 show the average grain yield and plant height of strains, their dispersion, extreme values and the correlation coefficients. Characteristics included in one table are excluded from the others.

Yield averages show that productivity is not the same in strains originating from different combinations, thus correlations can be studied at various

Table 1

Correlation between grain yield and plant height

Combination	n	Grain yield dag.			Plant height cm			
		$\bar{x}$	s	extreme values	$\bar{x}$	s	extreme values	r
				1965				
Bezostaya 1 × H. 1444 F_3	23	22	5.15	15—35	95	8.35	77—107	0.16^{NS}
Bezostaya 1 × Etoile de Choisy F_4	19	22	2.54	19—28	87	5.41	75—96	0.58***
(Bezostaya 1 × Bánkuti 1201 F_1) × Produttore 6 F_4	25	22	3.47	16—29	94	5.71	81—104	0.48***
Skorospelka 3 × Produttore 6 F_5	42	17	2.06	14—21	79	7.88	68—94	0.60***
Bez. 1 × San Pastore F_5	74	18	2.99	14—27	81	6.24	70—94	0.41***
				1966				
Bezostaya 1 × H. 1444 F_4	53	29	4.48	17—43	115	6.19	93—129	0.28**
Bezostaya 1 × Etoile de Choisy F_4	22	32	3.99	25—40	106	7.80	95—118	-0.15^{NS}
Bezostaya 1 × Produttore 6 F_5	17	29	3.00	23—34	93	9.48	83—109	0.15^{NS}
Bezostaya 1 × San Pastore F_6	20	28	4.08	22—34	92	8.32	80—108	0.40*
Skorospelka 3 × H. 1444 F_4	31	29	6.20	17—42	110	9.99	101—127	-0.01^{NS}

*** significant at P = 99%
** significant at P = 95%
* significant at P = 90%
^{NS} non-significant

yield levels. In 1965 strains selected from the following combinations were the best: (Bezostaya 1 × Bánkuti 1201 F_1) × Etoile de Choisy; Bezostaya 1 × 0.24—279; Bezostaya 1 × Etoile de Choisy; Bezostaya 1 × San Pastore; and Bezostaya 1 × Produttore 6; in 1966 Bezostaya 1 × H.1444, Skorospelka 3 × H.1444, and Bezostaya 1 × Mironovskaya 808; in 1967 Bezostaya 1 × Fertődi 293, Skorospelka 3 × H.1444, Bezostaya 1 × Mironovskaya 808 and its reciprocal, as well as Bezostaya 1 × Mironovskaya 264.

Extreme values show that even within the combinations there are great differences in yield between the strains. In certain combinations grain yield of the best strains was twice as high as that of the poorest ones.

In most combinations average plant height of strains was similar to that of Bezostaya 1. In several combinations, however, it was higher than that, though even in these combinations plant height in the lower strains did not exceed that of the standard varieties.

Correlation coefficients between yields and plant heights of strains are generally positive, but not equally high in every combination and year.

In 1965 correlation was significant even at the P = 99 per cent level,

Table 2

Correlation of grain yield and plant height

1967

Combination	n	Grain yield dag.			Plant height cm			r
		$\bar{x}$	s	extreme value	$\bar{x}$	s	extreme values	
Bezostaya 1×Mironovskaya 808 F_4	69	28	3.61	18—35	107	9.22	87—120	0.41***
Bezostaya 1×Mv 65—07 F_4	96	25	3.83	13—32	109	9.40	71—134	-0.08^{NS}
Bezostaya 1×Mironovskaya 264 F_4	41	27	4.00	20—35	105	8.14	91—114	0.18^{NS}
Bezostaya 1×Fertődi 293 F_5	59	32	3.81	22—38	102	3.44	92—114	0.44***
Bezostaya 1× Produttore (h) F_5	71	29	2.64	22—39	93	8.42	71—113	0.25**
Bezostaya 1×Etoile de Choisy F_5	47	30	3.65	22—40	96	9.68	82—115	0.26*
Bezostaya 1×H. 1444 F_5	51	31	5.69	21—43	109	7.50	91—125	0.42***
(Bezostaya 1×Fertődi 293 F_1)×Bezostaya 1 F_4	82	32	2,95	25—42	93	4.19	84—114	0.26**
(Bezostaya 1×Etoile de Choisy F_2)×Bezostaya 1 F_4	55	29	2.77	20—35	94	6.94	87—105	0,36***
(Bezostaya 1×San Pastore F_2)×Bezostaya 1 F_5	44	29	2.73	23—33	91	5.90	82—104	0.22^{NS}

*** significant at P = 99%
** significant at P = 95%
* significant at P = 90%
^{NS} non-significant

in four out of the five combinations examined. No significant correlation was found in the combination of Bezostaya 1 × H.1444. In 1966 none of the five combinations examined showed any correlation.

In 1967 ten combinations were examined. In four of them correlations were of medium strength, being significant even at the level of P = 99 per cent. In three combinations no significant correlation was found. Strains originating from the combination of Bezostaya 1 × Etoile de Choisy were studied for three years. In the first year a medium strong correlation, significant at the P = 99 per cent level was found between grain yield and stalk height, which was, however, not proved in the next two years. Progenies of the Bezostaya 1 × H.1444 combination were also examined in every year but correlation between yield and plant height was found only in the third year.

The correlation coefficient was generally higher in combinations where planst were, on average, higher and the range of variation wider.

Correlation between the yields of "A" strains and the height of the mother plants was also examined.

Table 3

Correlation between the grain yield of "A" strains and the height of the mother plant

Combination		n	Height of previous years			r
			$\bar{x}$	s	extreme values	
		1965				
Bezostaya 1 × H. 1444	F_3	23	76	9.68	56—100	−0.43**
Bezostaya 1 × Etoile de Choisy	F_4	19	68	10.33	49—81	0.40*
(Bezostaya 1 × Bánkuti 1201 F_2) Produttore	F_4	25	70	8.58	53—86	0.21^{NS}
Skorospelka 3 × Produttore	F_5	42	61	9.57	47—76	-0.07^{NS}
Bezostaya 1 × San Pastore	F_5	74	65	7.51	51—79	0.03^{NS}
		1966				
Bezostaya 1 × Fertődi 293	F_4	56	89	10.92	71—105	0.16^{NS}
Bezostaya 1 × H. 1444	F_4	53	89	7.81	70—107	0.22^{NS}
Bezostaya 1 × Etoile de Choisy	F_4	22	82	4.00	71—92	-0.12^{NS}
Bezostaya 1 × Produttore 6	F_5	17	73	9.29	61—90	-0.13^{NS}
Bezostaya 1 × San Pastore	F_6	20	75	6.67	62—92	0.57***
Skorospelka 3 × H. 1444	F_4	31	83	8.81	69—100	-0.09^{NS}
(Bezostaya 1 × San Pastore F_2) × Bezostaya 1	F_4	78	72	8.19	60—92	0.26**
		1967				
Bezostaya 1 × Mironovskaya 808	F_4	69	110	10.75	83—126	0.82***
Bezostaya 1 × Mv 65—07	F_4	96	104	8.89	90—122	0.31***
Bezostaya 1 × Mironovskaya 264	F_4	41	104	7.42	91—120	0.47***
Bezostaya 1 × Fertődi 293	F_5	59	111	7.25	95—122	0.12^{NS}
Bezostaya 1 × Produttore (h)	F_5	71	96	10.95	78—120	0.32***
Bezostaya 1 × Etoile de Choisy	F_5	47	96	8.85	74—114	0.14^{NS}
Bezostaya 1 × H. 1444	F_5	51	110	8.25	88—130	0.63***
(Bezostaya 1 × Fertődi 293 F_1) × Bezostaya 1	F_4	82	90	8.20	76—109	0.11^{NS}
(Bezostaya 1 × Etoile de Choisy F_2) × Bezostaya 1	F_4	55	95	14.12	87—110	0.23*
(Bezostaya 1 × San Pastore F_2) × Bezostaya 1	F_5	44	91	6.39	79—102	0.09^{NS}

*** significant at P = 99%
** significant at P = 95%
* significant at P = 90%
^{NS} non-significant

Table 3 shows that mother plants were low on the average, especially in the first two years, as we endeavoured to isolate plants with short stalks from the population, while the extreme values show that their range of variation was wide. In certain combinations the difference between the lowest and the highest plants was as much as 40—44 cm.

Data presented in the table do not show unequivocally negative or positive correlations between the characteristics examined. In the Bezostaya 1 × H.1444 combination at P = 95 per cent, significantly negative, while in the Bezostaya 1 × Etoile de Choisy at P = 90 per cent significantly positive correlation was found in 1965.

In 1966 seven combinations were examined. In five combinations no significant correlation was found. Strains originating from the combination of Bezostaya 1 × San Pastore showed, however, a medium close positive correlation significant at P = 99 per cent. In the combination of (Bezostaya 1 × San Pastore) × Bezostaya 1 correlation was loose, being significant at P = 95 per cent.

In four out of the ten combinations examined in 1967 no correlation was found. In the combinations of Bezostaya 1 × Mironovskaya 808, Bezostaya 1 × H.1444, Bezostaya 1 × Mv 65—07, Bezostaya 1 × Mironovskaya 264 and Bezostaya 1 × Produttore (h) positive correlation, significant at P = 99 per cent was found. Correlation was especially close in the combinations of Bezostaya 1 Mironovskaya 808 and Bezostaya 1×H.1444. Data also show that correlations were found in combinations originating from crosses among plants of different heights, and the selected plants were, on average, high and had a wide range of variation. Combinations showing correlation between the yield and the height of the mother plant were partly identical with those showing correlation between yield and plant height.

Correlations between yield and plant height as well as between yield and height of the mother plant having been determined in certain combinations, correlation coefficients between the height of the mother plant and plant heights of "A" strains were also calculated. Results are shown in Table 4.

Table shows that plant heights in "A" strains examined in 1965 were, without exception, in a positive correlation with the heights of the mother plants.

Correlations of similar tendency, reliability and closeness were obtained in 1966.

In 1967 close correlation was found in the following combinations: Bezostaya 1 × Etoile de Choisy, Bezostaya 1 × Etoile de Choisy, Bezostaya 1 × Mironovskaya 808, (Bezostaya 1 × San Pastore) × Bezostaya 1 and Bezostaya 1 × Fertődi 293. Non significant correlation was found in Bezostaya 1 × Mironovskaya 264 only. In the combinations Bezostaya 1 × Produttore (h) and (Bezostaya 1 × Etoile de Choisy) × Bezostaya 1 correlation among plant heights over the two years was very loose. On the other hand, plants in the last two combinations are on the average small.

Thus, in the great majority of combinations a close correlation was found in plant height between "A" strains and mother plants.

Correlation between grain yield and growth season was studied subsequently. The length of the growing season was determined in 1965 and 1967 by the time of earing, while in 1966 by the time of ripening. Results are presented by Table 5.

The table shows both the average and the extreme earing (ripening) times of combinations. Out of the five combinations examined in 1965 only

Table 4

Correlation in plant height between mother plants and "A" strains

Combination		n	Height			r
			$\bar{x}$	s	extreme values	
		1965				
Bezostaya 1 × H. 1444	F_3	23	76	9.7	56—100	0.52***
Bezostaya 1 × Etoile de Choisy	F_4	19	68	10.3	49—81	0.60***
(Bezostaya 1 × Bánkuti 1201 F_1) × Produttore	F_4	25	70	8.6	53—86	0.67***
Skorospelka 3 × Produttore 6	F_5	42	61	9.6	47—76	0.67***
Bezostaya 1 × San Pastore	F_5	74	65	7.5	51—79	0.62***
		1966				
Bezostaya 1 × H. 1444	F_4	53	115	6.2	93—129	0.65***
Bezostaya 1 × Etoile de Choisy	F_4	22	106	7.8	95—118	0.76***
Bezostaya 1 × Produttore 6	F_5	17	93	9.5	83—109	0.42*
Bezostaya 1 × San Pastore	F_6	20	92	8.3	80—108	0.78***
Skorospelka 3 × H. 1444	F_4	31	110	10.0	101—127	0.75***
		1967				
Bezostaya 1 × Mironovskaya 808	F_4	69	107	9.2	87—120	0.81***
Bezostaya 1 × Mironovskaya 264	F_4	41	105	8.1	91—114	0.14^{NS}
Bezostaya 1 × Fertődi 293	F_5	59	102	3.4	92—114	0.78***
Bezostaya 1 × Produttore (h)	F_5	71	93	8.4	71—113	0.22*
Bezostaya 1 × Etiole de Choisy	F_5	47	96	9.7	82—115	0.88***
Bezostaya 1 × H. 1444	F_5	51	109	7.5	91—125	0.52***
(Bezostaya 1 × Fertődi 293 F_1) × Bezostaya 1	F_4	82	93	4.2	84—114	0.74***
(Bezostaya 1 × Etoile de Choisy F_2) × Bezostaya 1	F_4	55	94	7.0	87—105	0.25**
(Bezostaya 1 × San Pastore F_2) × Bezostaya 1	F_5	44	91	5.9	82—104	0.79***

*** significant at P = 99%
** significant at P = 95%
* significant at P = 90%
^{NS} non-significant

Bezostaya 1 × San Pastore displayed a very loose negative correlation. Out of the six combinations examined in 1966 a positive, loose but significant at P = 99 per cent correlation was found in two combinations: Bezostaya 1 × Etiole de Choisy and Bezostaya 1 × Produttore 6.

In four of the ten combinations studied in 1967, namely: Bezostaya 1 × Mironovskaya 808, Bezostaya 1 × Mv 65—07, Bezostaya 1 × Mironovskaya 264 and Bezostaya 1 × Produttore (h), medium close and loose — though significant at P = 99 per cent correlations were found. These are combinations where the pollen parent belongs to a variety several days later than that of the seed parent, and the range of hybrid variation is wide.

There was a very loose positive correlation also in the combinations (Bezostaya 1 × Fertődi 293) × Bezostaya 1 and Bezostaya 1 × Fertődi 293. In four combinations no significant correlation in any directions was found.

Table 5

Correlation of yield and growth season

Combination		n	Growth season			r
			$\bar{x}$	s	extreme values	
			1965			
Bezostaya 1×H. 1444	F_3	23	June 10	3.51	June 3—June 15	0.03^{NS}
Bezostaya 1×Etoile de Choisy	F_4	19	June 3	1.06	June 1—June 5	-0.10^{NS}
(Bezostaya 1×Bánkuti 1201 F_1) ×Prod. 6	F_4	25	June 6	2.61	June 2—June 12	0.09^{NS}
Skorospelka×Produttore 6	F_5	42	June 4	1.83	June 1—June 9	-0.19^{NS}
Bezostaya 1×San Pastore	F_5	74	June 8	1.30	June 5—June 10	—0.23**
			1966			
Bezostaya 1×H. 1444	F_4	53	July 11	2.18	July 6—July 14	0.03^{NS}
Bezostaya 1×Etoile de Choisy	F_4	22	July 6	1.55	July 3—July 9	0.44**
Bezostaya 1×Produttore 6	F_5	17	July 6	2.04	July 4—July 10	0.45**
Bezostaya 1×San Pastore	F_6	20	July 4	1.31	July 2—July 8	0.09^{NS}
Skorospelka 3×H. 1444	F_4	31	July 9	2.65	July 4—July 13	0.19^{NS}
(Bezostaya 1×San Pastore F$)_2$× Bezostaya 1	F_4	78	July 6	2.25	July 3—July 9	0.06^{NS}
			1967			
Bezostaya 1×Mironovskaya 808	F_2	69	May 24	2.72	May 18—May 29	0.34***
Bezostaya 1× Mv 65—07	F_4	96	May 23	2.89	May 19—May 31	0.56***
Bezostaya 1×Mironovskaya 264	F_4	41	May 24	3.08	May 18—May 30	0.40***
Bezostaya 1× Fertődi 293	F_5	59	May 19	1.88	May 14—May 25	0.22*
Bezostaya 1×Produttore (h)	F_5	71	May 21	1.74	May 16—May 24	0.33***
Bezostaya 1×Etoile de Choisy	F_5	47	May 21	2.09	May 18—May 28	0.14^{NS}
Bezostaya 1×H. 1444	F_5	51	May 26	3.06	May 18—June 5	-0.03^{NS}
(Bezostaya 1× Fertődi 293 F_1)× Bezostaya 1	F_4	82	May 19	1.67	May 15—May 25	0.22**
(Bezostaya 1×Etoile de Choisy F_2)×Bezostaya 1	F_4	55	May 22	1.68	May 20—May 25	-0.09^{NS}
(Bezostaya 1×San Pastore F_2)× Bezostaya 1	F_5	44	May 21	1.44	May 18—May 25	-0.04^{NS}

*** significant at P = 99%
** significant at P = 95%
* significant at P = 90%
^{NS} non-significant

Conclusions

In "A" strains originating from the combinations examined no consistent correlation was found in the different years. However, when combinations were studied separately, several characteristics were found to be either positively or negatively correlated with the yield.

Frequency of correlations between grain yield and plant height in "A" strains as well as between the grain yield of "A" strains and the height of the mother plant suggest that plant height may indicate productivity in certain combinations.

The close correlation between plant heights of mother plants and "A" strains in most cases shows the inheritable nature of plant height, in spite of the fact, that mother plants were studied in sparsely spaced, while "A" strains in dense stand. Therefore, in combinations showing a correlation between the grain yield of strains and the plant height the latter can be accepted as a basis for selection, that is, plant height may be a suitable indicator of productivity.

It should be added, that correlation between grain yield and plant height is a peculiarity characteristic of the combination. It generally occurs in combinations obtained by crossing plants of different height.

Naturally, selection through plant height is possible only in combinations where the taller types are also suitable for breeding purposes. Conclusions drawn concerning the positive correlation of plant height and grain yield correspond with results obtained by CLARK—HOOKER (1926), TORRIE (1936) and LEGRAND (1966).

Data on the grain yield and growth season of strains suggest that under Hungarian conditions late types are not more productive. This statement does not contradict the loose positive correlation found in 1967 in four combinations, since no really late plants were included in these populations.

After all, it is selection of early plants from the examined material that seems to be reasonable, since earliness is an agronomic advantage under Hungarian conditions and does not mean any disadvantage in productivity either.

Our results confirm LUKYANENKO's findings (1966) but contradict observations made by CLARK—HOOKER (1926), BHIDE (1963) and LEGRAND (1966). This contradiction is caused by the fact that wheat types grown succesfully in different experimental sites are not the same. Under Hungarian conditions, just as at Krasnodar, early varieties ripen before the summer drought can be successfully grown. Late varieties are not able to display their productivity, as thousand-grain-weight cannot fully develop in them.

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CHLORIDE ION CONTENT IN NORMAL AND PATHOLOGICAL EWE-MILK

By

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The paper deals with the chloride ion content of normal and pathological ewe-milk subjected to the Schalm-test, mercurimetric titration and biometrical examination, and determines an average of 77 mg% for normal and a limit of 140 mg% or over for pathological ewe-milk.

Introduction

Few data are presented in the literature on the chloride ion content of ewe-milk.

The earliest information on chloride ion content was given in 1881 by Weiske and Kennepohl (cit. Grimmer 1910) who on the basis of the chloride percentage in the ash content determined by them found it to be 55.8 mg%. Later Rievel—Fettick (1909) found 70.9 mg%, while Abderhalden (cit. Grimmer *et al.* 1930) 129.7 mg%. The most recent data are those of Droese—Stolley (1960), they determined 71.0 mg %, while our Station in the course of investigations 85.0—175.0 mg% as the value of chloride ion content.

It is not only because of the low number of literary data but also due to the diagnostical significance of the subject that the presentation of the successively obtained data is so important, as investigations have revealed that demonstration of increase in the chloride ion content may be a complementary test in diagnosing mastitis not only in cattle (Redaelli—Nani 1957, Hauke—Schönherr 1963) but in sheep too, as proved by the correlation coefficient of 0.85 at 0.1 P% obtained in comparison with the Schalm-test after 39 measurements.

The latter findings were confirmed from a different aspect when Berke (1933) pointed out that the biochemical properties of cow's milk and ewe-milk, and their biological and pathological changes are qualitatively similar.

It must also be taken into consideration, of course, that at the beginning and end of lactation the chloride ion content of ewe-milk is higher than in the middle of lactation, and in order to obtain a perfect diagnosis chloride ion content must be determined in milk originating from the other half udder too.

Another reason why it is so important to clear up this question is that with the increased application of the mechanical milking of ewes a growing number of mastitis cases and the related danger of food poisoning (staphylococcosis) can be reckoned with. That is why beside the clinical examinations improvement and simplification of laboratory diagnostical methods are so important.

The above opinion was confirmed by our investigations in which 9 (0.87 per cent) of 1032 hand milked, and 34 (6.20 per cent) of 532 mechanically milked ewes proved to suffer from mastitis.

Material and Method

In each test milk originating from one half udder of a single ewe was used.

Chloride ion examination is performed with the Volhard method (cit. ERDEY 1966) all over the world. This method, however, requires relatively high quantities of milk (50 ml for each test), is complicated, difficult to evaluate, and therefore unsuitable for examinations of whole flocks.

There are more up-to-date methods of chloride ion determination, e.g. amperometric, potentiometric, coulombometric titrations, etc. (KACSKOVICS—SCHUMANN 1968), they have high instrument, space and condition requirements, however.

The method most suitable for the present study is mercurimetric titration (PAZDERKA—RADEMACHER—KRÁLOVE 1967) which — according to ERDEY (1966) — is based on the phenomenon of Hg_2^{2+} and Hg^{2+} ions in nitric media reacting to diphenylcarbazide dissolved in alcohol (ethanol) either with violet colouration or with blue precipitation depending on their quantities. This reaction is inhibited by the chloride ions.

Description of the test. 1 ml of the sample is placed in a test-tube containing 4 ml 1.25 per cent nitric acid, shaken up, then two drops of ethanol–diphenylcarbazide $(O{=}C/NH \cdot NH \cdot C_6H_5)_2$, (0.5 g/100 ml ethanol) are added, shaken up again, then from a microburette or pipette of 0.01 ml scale titrated with 0.0141 n mercury-nitrate $(HgNO_3)_2$ solution until the content of the test-tube changed from opaque white into a pale lilac colour. Mercury-nitrate solution of 1.0 factor reduced by 1 ml corresponds to 100 mg% chloride ion.

Results were evaluated on the basis of SVÁB's (1967) biometric method, by calculating arithmetic means, dispersion and significance.

Chloride ion content was compared with the result of the Schalm-test which had been proved in our previous paper to be suitable for detecting mastitis in sheep.

Results

The results of chloride ion determination are presented in Tables 1 to 5.

Discussion

Chloride ion results of ewe-milk showing negative reaction to the Schalm-test generally agree with the results published so far. Chloride ion content in ewe-milk giving 1+, 2+, 3+ reactions is remarkably high compared to the negative milk samples, therefore mastitis or pathological changes in milk are not difficult to diagnose when the biological, pathological and epidemiological conditions of the flock are known. Diagnostical difficulties are caused by milk

Table 1

Chloride ion content in milk giving negative reaction to Schalm-test

X_i mg%	f_i observation	f_iX_i total mg%
57	6	342
58	1	58
60	4	240
67	3	192
67	6	402
71	8	568
75	2	150
78	5	390
82	5	410
85	3	255
89	4	356
96	2	192
99	1	99
103	3	309
110	1	110
113	2	226
121	1	121

$$n_1 = 57 \qquad \Sigma f_i X_i = 4420 \quad \overline{X}_1 = \frac{\Sigma f_i X_i}{n_1} = 77 \text{ mg\%}$$

Table 2

Chloride ion content in milk giving uncertain reaction to Schalm-test

X_i mg%	f_i observation	f_iX_i total mg%
127	1	127
138	1	138
140	1	140
149	1	149
152	1	152

$$n_2 = 5 \qquad \Sigma f_i X_i = 706 \qquad \overline{X}_2 = \frac{\Sigma f_i X_i}{n_2} = 141 \text{ mg\%}$$

Table 3

Chloride ion content in ewe-milk giving 1+ reaction to Schalm-test

X_i mg%	f_i observation	f_iX_i total mg%
154	1	154
160	1	160
163	2	326
170	1	170
172	4	688
174	1	174
175	3	525

$$n_3 = 13 \qquad \Sigma f_i X_i = 2197 \qquad \overline{X}_3 = \frac{\Sigma f_i X_i}{n_3} = 169 \text{ mg\%}$$

Table 4

Chloride ion content in ewe-milk giving 2+ reaction to Schalm-test

X_i mg%	f_i observation	f_iX_i total mg%
172	1	172
174	1	174
175	2	350
195	1	195
202	1	202

$$n_4 = 6 \qquad \Sigma f_i X_i = 1093 \qquad \overline{X}_4 = \frac{\Sigma f_i X_i}{n_4} = 182 \text{ mg\%}$$

Table 5

Chloride ion content in ewe-milk giving 3+ reaction to Schalm-test

X_i mg%	f_i observation	f_iX_i total mg%
258	1	258
293	1	293

$$n_5 = 2 \qquad \Sigma f_i X_i = 551 \qquad \overline{X}_5 = \frac{\Sigma f_i X_i}{n_5} = 275 \text{ mg\%}$$

samples giving uncertain reactions; the limit value must be around the average of milk samples giving uncertain and 1+ reactions (Tables 2, 3).

Mean value of data contained in Tables 2 and 3 on the basis of the formula

$$\bar{X} = \frac{\sum f_i x_i}{n_2+n_3} \text{ is } 155.0 \text{ mg } \%.$$

Sum of squares of deviations

$$SQ_{2+3} = \sum X_i^2 - \frac{(\sum X_i)^2}{n} = 214.05.$$

Standard deviation:

$$S_{2+3} = \sqrt{\frac{SQ_{2+3}}{n_2+n_3-1}} = 14.62, \text{ which seems right because}$$

$\bar{X} \pm S_{2+3} = 155 \pm 14 = 141,\ 169$ mg %, which corresponds to the values of $\bar{X}_2$, $\bar{X}_3$.

Deviation of differences:

$$s_2^2 = \frac{SQ_2}{n_2 - 1} = 335.75$$

$$S_3^2 = \frac{SQ_3}{n_3 - 1} = 191.33$$

$$S_d = \sqrt{\frac{S_2^2}{n_2} + \frac{S_3^2}{n_3}} = 9.04.$$

Significance analysis:

$$\text{value of } t = \frac{\bar{X}_2 - \bar{X}_3}{S_d} = 3.09.$$

Degree of freedom: (FG) $= n_2 + n_3 - 2 = 16$
$P = 1\%$.

There is a significant difference of 99% probability between the data of Tables 2 and 3.

According to the data of the calculations and tables, as well as other data taken in consideration, chloride ion contents over 140 mg % in milk obtained from one half under suggest mastitis, or some other pathological change of the milk.

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THE MACRO- AND MICROELEMENT CONTENT OF THE FLOWER

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The authors studied the macro- and microelement contents of flowers in petals and carpels separately. On the basis of quantitative analyses they tried to find an explanation for the differentiated importance of various elements in fruit formation. The macroelements (except magnesium) as well as iron and manganese of the microelements were found to decrease while copper and zinc intensively increase in the flower as compared to the vegetative organs. Fluctuations in the surprisingly low ratios of Mn/Cu and Mn/Zn were caused in most cases by differences in copper and zinc contents rather than by those in manganese contents.

Introduction

Beside the wide-spread practice of supplying macroelements by fertilization the necessity for combining fertilizers with microelements has recently been a subject of frequent discussion. Long ago researchers called attention to the physiological importance of microelements (found in the periodic table at some distance from the "line of the nutritive elements") which occur at low concentrations in plants. This does not, naturally, mean that the biochemical background of this subject has been sufficiently elaborated, and for some time the solution of long-term objectives in plant production has partly been based on this work. It has been proved, namely, that under favourable conditions the microelements may promote the improvement of qualitative and quantitative indices of yields. A wide range of publications deal with the role of manganese (Klimov–Klimova 1966, Vlasyuk–Kovaltschuk 1967, Rajapakse–Norton 1968, Toms 1968, Tölgyesi 1969, Vlasyuk–Klimovitzkaya 1969), zinc (Jelenkovic 1966, Masev 1966, Patarinski 1967), copper (Pejve 1967, Alekseev–Polyakov 1968, Andreev–Mihaylitschenko 1968), molybdenum (Avdonin–Arens 1966, Klimov–Klimova 1966, Nikiskina–Medvedeva 1966, Godnev–Lesina 1967, Petkov 1968, Boswell–Anderson 1969) and other microelements in plant metabolism.

Appropriate reinforcement of deficient microelement reserves in the soils of Hungary can be carried out only if sufficient analytical data are available. The proper way is to complete the microelement analysis of the soil with the analytical results of the cultivated plants in question. Accordingly, in the course of summarizing the subject in some detail, **Spector** (1956) and **Tölgyesi** (1969) discussed thoroughly the mineral composition of various plant organs excluding the flower. Spector — while mentioning the flower — touched upon the question but slightly. Since experiments concerning this subject have hardly been performed it is easy to understand why such results are missing from the text-books and special reviews. We think, however, that the idea of realizing this work is not only justified by a demand on completeness, it is also considered a further step in the study of fruit formation.

Material and Method

When collecting material for the examinations we endeavoured to include both herbaceous and ligneous plants in the samples. In the tables showing the macro- and microelement contents of flowers petals and carpels are grouped separately. Flowers were collected immediately before the dehiscence of anthers, so the analytical data of the anther can be considered as of full value.

Samples were dried for eight hours at 60 °C in an exsiccator, then pulverized in a braying mortar. The required amount of dry matter thus obtained was reduced to ashes in a heating furnace. Samples were then placed into a mixture of cc. HCl, cc. HNO_3 and distilled water of a ratio of 1 : 1 : 8 and boiled to make a more perfect solution. The decoction was ultimately diluted with distilled water to a 20 ml stock-solution with an acid concentration of about 2 per cent. Ca, Mg, Na, K, Fe, Mn, Zn and Cu contents were determined with a Perkin-Elmer 290 B.-type atom-absorption spectro-photometer.

Results

The introduction in which — as a contrast to the title — the special literature on microelements is dealt with exclusively and therefore may be considered as one-sided by many will be easily understood when the results have been evaluated. We should like to make it clear that it is worth paying an attention to the microelements — as well as the macroelements — from the point of view of the development and the final state of development in the reproductive organs.

In the course of analysing the macroelements of the flower we found that potassium and calcium contents were lower than in the foliage leaves and higher than in the seed, while sodium showed an increasing tendency. The latter result was very surprising. It can also be clearly seen from Table 1 that the magnesium content of flowers is sufficiently balanced as compared to magnesium fluctuations in the vegetative organs. Calcium accumulates primarily in the sepals and stamina, while in the pistil displays a very low percentage occurrence. On the basis of the analyses on the flower the high potassium content of papilionaceae is well defined too.

As compared to the microelement level of vegetative organs — first of all foliage leaves — the microelements concerned can be classified into two

Table 1

Macroelement composition in flowers of various plant species

Sample	Ash content % g/100 g	K	Ca	Mg	Na
		g/100 g			
Linden					
bract	6.60	0.64	0.58	0.17	
sepal	8.91	1.03	0.78	0.22	
petal	8.53	1.07	0.63	0.20	
stamen	7.97	0.80	0.83	0.26	
pistil	5.52	0.70	0.40	0.16	
Horse bean					
sepal	7.53	3.82	0.89	0.47	
standard	5.08	2.53	0.31	0.33	
wings	5.72	3.00	0.32	0.46	
keel	5.14	2.60	0.38	0.39	
stamen	7.99	4.10	0.56	0.50	
pistil	7.19	3.53	0.34	0.42	
Pea					
sepal	6.65	1.25	1.64	0.18	0.11
standard	5.69	1.69	0.59	0.24	0.13
wings	5.52	1.70	0.48	0.23	0.07
keel	5.22	1.88	0.96	0.36	0.19
stamen	5.44	1.26	1.08	0.20	0.12
pistil	6.92	1.93	0.31	0.21	0.05
Sunflower					
pollen	2.62	0.20	0.15	0.07	
Hazel					
pollen	3.85	0.59	0.17	0.14	

groups. One of the groups includes iron and manganese, the other copper and zinc, with a decreasing and increasing (or stagnating) tendency, respectively. As a consequence, flowers of the plants presented are characterized by essentially different proportions of microelements, as expressed by the Mn/Zn ratio almost regularly lower than one, and often similarly low ratio of Mn/Cu (Table 2). It is interesting that the differences between these low values are generally caused by fluctuations in the copper and zinc contents rather than in the manganese content. When comparing the floral organs in these three plants we find an increased copper content in the carpels. The microelement accumulation in the pollen is accompanied by an even more intensive decrease in the macroelements mentioned (Tables 1 and 3).

Table 2

Microelement content of pollen in various plant species

Samples	microelements Mn			Fe			Zn			Cu		
	plant species											
	L	P	H	L	P	H	L	P	H	L	P	H
Bract	10			156			8			3		
Sepal	12	62	28	85	140	330.8	9	59	89	4	24	48
Petal	11			100			23			4.6		
Standard		37	25.9		160	151.5		68	52		48	48
Wings		36	29.5		140	192.6		60	57.6		24	50
Keel		55	28		170	129.8		74	52		30	37
Stamen	15	36	26.2	40	100	224.9	9	48	86.2	5	20*	75
Pistil	7	27	19.1	30	110	138.2	9	94	78.7	6	39	69

Note: Results are given in mg/kg.
Signs and abbreviations used:
L = linden
P = pea
H = horse bean
* = without pollen

Table 3

Microelement content of pollen in sunflower and hazel

Sample	Fe	Cu	Mn	Zn
	mg/kg			
Sunflower	50	20	8	30
Hazel	220	31	46	80

Conclusions

The most general conclusion drawn from the results obtained is the macroelement content being essentially lower in the flower than in the vegetative organs. With magnesium excluded this decrease is further differentiated in relation to the carpels, and probably the generative cells, although no data concerning the egg-cells are available.

Among the microelements mentioned the decreasing, stagnating or increasing tendency of the iron–manganese and copper–zinc group compared with the vegetative parts can also be considered a fundamental difference.

On the basis of our knowledge acquired so far we are trying to give some explanation for the relation of manganese and copper. Manganese can be brought into connection primarily with photosynthesis, namely, the photolysis of water is a process of high manganese requirements, but its presence is also indispensable in protein and fat metabolism. During respiration the oxidase–peroxidase reaction similarly requires manganese ions. According to Tölgyesi (1969) the carbohydrate content changes parallel with the manganese content which confirms its close connection with the synthetic processes of plants. Going a step further we can say that the same trend of differences in photosynthetic intensity and manganese content between the plant organs may be a proof of the differentiated biological importance of microelements. The role of copper in the system of assimilation and dissimilation seems "more balanced". The copper demand of the Hill-reaction (plastocyanine), and the accumulation of copper in the chloroplasts equally confirm the important role played by copper in photosynthesis. On the other hand, the members of the oxido-reduction enzyme system beyond the mitochondrial respiration chain, the polyphenoloxidase and ascorbic acidoxidase are all enzymes containing copper. In some authors' opinion copper determines the proteolytic activity too. It is known that, as a consequence of an "explosion-like" growth, respiration becomes still more intensive in the flowers with a gradual decrease in the following order of succession: pistil, stamina, petals, sepals.

The similar trend of the copper content in the floral organs also seems to support its significant role in respiration and protein metabolism.

The relatively high sodium level is difficult to explain, it requires further studies.

Naturally, quite a number of open questions are still to be answered in order to make the future application of microelement fertilization efficient. Our investigations form an integrate part of our experiments on radioactive radiation stimulation, namely, in our opinion, stimulation is in connection with the nutrient uptake of plants. Increasing the permeability of the cell-wall between certain limits, in combination with adequate methods of foliage spray application (development stage, ratio of mixing, specific features of plants, etc. taken in consideration) may promote the improvement of quantitative and qualitative yield indices probably by increasing the nutrient uptake.

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THE EFFECT OF ADDED DL-METHIONINE IN DEFICIENT DIETS FOR GROWING CHICKS ON GROWTH AND EFFICIENCY OF FEED UTILIZATION

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Feeding experiments with Hungarian chicks on different levels of sulphur amino acid in diets were made in order to find out the suitable requirements. This study includes 160 chicks (48 males and 112 females) taken from the Farm of Faculty of Agriculture, Keszthely, Hungary. The birds were divided into three groups. While Group I received the basal ration, for Groups II and III DL-methionine was added, the total sulphur amino acid in the diet were 0.9 and 1.2 per cent, respectively. The period of this experiment started when the chicks were 21 days old and terminated at the 81 days of age, divided into four periods lasting 15 days each. The results obtained are as follows: The quantitative requirement of the chicks for sulphur amino acid during the period from 21—66 days of age was more than 0.62 and less than 1.2 per cent being 0.90 per cent in the diet to support maximum feed conversion and maximum growth for both sexes. The excess of sulphur amino acid has a depressant effect on growth and efficiency of feed utilization. The sulphur amino acid requirement was higher in the early growing period of the chick than that of the older one of the same breed, due to the relatively more surface area per unity of body weight and the development of the feather. The addition of DL-methionine to the deficient diet at a rate of 0.90 per cent improved the feed efficiency even though there were no differences in daily gain, except in the third period (51—66 days of age) when the daily gain also increased.

Introduction

The protein in the ration of chick must be balanced in essential amino acids to insure a maximal growth and efficiency of feed utilization. The amino acid requirement will vary with the level of protein fed, the relative proportions of amino acids are more important than the protein level. Therefore, the requirement of a bird for a given amino acid is not a definite figure but it varies with the level of other amino acids in the diets. The demand for some of the amino acid is expressed as percentage of the protein fed, the value decreases as the protein level increases. In most instances diets formulated for the chick are deficient or limited in one or more of essential amino acids.

Several attempts have been made in the field of poultry nutrition to find out the proper requirement of methionine in the diet for growing chickens. It is considered necessary to inter-relate the scattered knowledges in order to realize the variations in the recommended values and their wide ranges. MCKITTRICK (1947), GLISTA (1951), SAXENA—MCGINNIS (1952) and REED *et al.* (1954) recommended 0.5 per cent methionine in the diet, the value of DEAN—SCOTT (1962) was slightly higher being 0.55 per cent.

Adams *et al.* (1962) stated a level of 0.5 per cent sulphur amino acid appearing to be adequate to support maximal growth of 4-week-old chicks.

McGinnis—Evans (1947), **Milligan** *et al.* (1951) and **West** *et al.* (1951) reported sulphur amino acid requirement of 0.71, 0.72 and 0.74 per cent, respectively, whereas **Almquist** (1952), **Hill** (1953), the National Research Council — N.R.C. (1960) and **Dean—Scott** (1962) recommended the sulphur amino acid requirement to be 0.8 per cent in the diet.

Other workers, **Briggs** *et al.* (1942), **Grau—Kamei** (1950) and **Jukes—Stokstad** (1951) suggested that 0.90 per cent of sulphur amino acid in the diet was a suitable value for maximal feed efficiency and growth.

The result of **Lewis** *et al.* (1951) showed that the requirement of sulphur amino acid was higher, being 1.18 per cent of the diet.

Recently **Nelson** *et al.* (1960) have showed that the quantitative requirement of chicks for sulphur amino acids is more than 3.3 and less that 3.81 approximately 3.5 per cent of protein in the diet.

It seems that several factors are involved and other than the requirement of the methionine e.g. the age of the chick, as it is possible that the sulphur amino acid requirement may be more intense in young fowls that have relatively more surface area per unit of body weight.

In this connection **Levis** *et al.* (1951), **Menge** *et al.* (1953), **Hill** (1953) and **Bolton** (1963) reported that the supplement of DL-methionine was of value in improving growth and feed efficiency in the early growing period of chick but this disappeared at the time when broilers reached the market age.

Another aspect of this general problem, the genetic background of the bird as a factor influencing a precise requirement for the methionine is clearly evident from the reports of **Hess** *et al.* (1962), **Nesheim—Hutt** (1962), **Hess** (1963), **Gorden** (1963), **Enos—Moreng** (1964) and **Nesheim** *et al.* (1964) who established lines differing in body weight at 3 weeks of age by selection on methionine deficient diet. **Miller** *et al.* (1960) showed a breed difference in the rate of methionine conversion to cystine. **Mc Donald** (1957, 1958) demonstrated a breed difference between white Leghorn and Black Australops chicken in utilization of methionine and cystine.

It was realized that the testing of the proper level of methionine be made with the supplementation of crystalline DL-methionine to the rations because increasing total protein in the diets implies serious protein wastage and also the providing of several protein sources results in an increased total cost of the feed.

Therefore, the purposes of experiments were to study the effect of different levels of DL-methionine in the diets to find the most suitable requirements for maximal growth and feed efficiency at different ages of white Leghorn chickens commonly bred in Hungary.

Material and Method

Chickens used in experiments were taken from the Farm of Faculty of Agriculture, Keszthely, Hungary in May 1966. The number of chickens was 160, 48 males and 112 females. The experiment of 60 days duration started at the 21 days of age and continued till the chickens were 81 days old. The duration of the experiment was divided into four periods lating 15 days each.

The chickens were housed in electrically heated battery brooders with wire mesh floors. Each chick was marked with a numbered wing band and weighed per two weeks thereafter early in the morning prior feeding on sensitive balance before each period and at the end of the experiment.

Feed and water were offered ab libitum and a daily record of feed consumption was kept. The composition of the basal diets used in the experiment is given in the following table:

Ingredient	%	Protein mg	Methionine g	Cystine g	Total sulphur amino acid g	Total digestible nutrients (T.D.N.) g
Ground maize	40.0	3.98	0.072	0.060	0.132	29.28
Ground wheat	20.0	2.02	0.040	0.040	0.080	14.30
Soybean meal	13.0	5.03	0.065	0.065	0.130	4.78
Peanut meal	13.0	5.29	0.071	0.071	0.140	6.98
Wheat bran	6.0	0.85	0.010	0.010	0.020	2.62
Lucerne dried	1.4	0.23	0.004	0.003	0.007	0.56
Fish meal	4.0	2.60	0.064	0.048	0.112	2.76
Total	97.4	20.00	0.324	0.297	0.621	61.28

The ration was supplemented by minerals, salt and vitamins. The Total Digestible Nutrients (T.D.N.) of this ration was 61.28 per cent as feed, the total crude protein was 20.00 per cent as feed. These values of the protein and T.D.N. of the ingredients were practically obtained in a previous work by the author (in press) except in case of the fish meal, the data were calculated according to Titus (1961).

The analytical method i.e. the paper chromatography for amino acid determination established by Richard *et al.* (1960) was applied to determine the methionine and cystine in the feed-stuffs.

The chickens have been divided into three groups. Group I (12 males and 28 females) received the basal ration deficient in sulphur amino acids being 0.62 per cent of the diet to study the effect of the deficient sulphur amino acids on growth and feed efficiency. Chicks in Group II (18 males and 42 females) were reared on the basal ration with added DL-methionine at a rate of 0.28 per cent to reach a level of 0.90 per cent total sulphur amino acid. In Group III (18 males and 42 females) a higher level of DL-methionine was added to the basal ration in order to study the effect of excess sulphur amino acids on growth and efficiency of feed utilization, the total sulphur amino acids offered to this group being 1.20 per cent of the diet.

The average weight has been calculated. The daily gain, growth measure (daily total digestible nutrients consumed in grams; daily body weight gain in grams) and relative growth rate (the final body weight in grams — the age of the bird in days) were estimated after Broody (1959) and Darwish *et al.* (in press).

In order to evaluate the differences obtained among the groups at separated and combined intervals a Student's "t" test was carried out according to Snedecor (1956).

Results

The results in Table 1 showed that the average daily gain of male chicks was nearly the same in the first period (21—36 days of age) and the second period (36—51 days of age) in Group I and Group II. In the third period (51—66 days of age), the average daily gain was higher in Group II than the corresponding one in Group I which were of 36.6 ± 1.47 and 31.5±1.22 g, respectively. The increase in the average daily gain was significant ($0.05 > P > > 0.02$).

During the fourth period (66—81 days old) the average daily gain was noticeably higher in Group I than in Group II which were 39.9± 4.87 and 36.1± 4.92 g, respectively.

The average daily gain of birds in Group II, during the four period, was higher than in Group III.

On the other hand, general improvement in feed conversion was observed in Group II during the first, second and third periods, 1.43, 1.49 and 1.80 growth measure stated, respectively. The corresponding values of growth measure of Group I were higher being 1.46, 1.55 and 2.17. The higher variation between the two groups and being occurred in the third period was 21 per cent more.

In the last period the average growth measure was slightly higher in Group II than in Group I.

Comparing the data of the growth measure between Group II and Group III, the average growth measure during the four periods was lower in Group II than in Group III.

It is obvious from Table 2 that the average daily gain of female chicks was higher in Group II than in Group I during the first, second and third periods. In the last period the average daily gain in Group I was 30.5 ± 1.93, higher than that of Group II which was 24.2 ± 1.56. The increase was significant $P < 0.05$.

The average daily gain (except the last period) was higher in Group II than in Group III.

The efficiency of feed utilization in Group II was better than that of Group I and Group III during the first, second and third periods. In the fourth period this improvement of feed conversion (in Group II) disappeared.

The results of the whole experimental period (60 days, from 21 days to 81 days old) given in Table 3, indicated that the average daily T.D.N. consumed by Group II was less than in the other two groups. The average daily gain of male chicks in Group II was slightly higher than in Group I, but there was a great difference between Group II and Group III. In case of the female, the average daily gain in Group I was higher than both in Group II and Group III.

Table 1

Average daily requirements, daily gain and growth measure among different groups of male chickens in the four periods

Group	I				II				III			
Period	1	2	3	4	1	2	3	4	1	2	3	4
Total Digestible Nutrients (T.D.N.) gms.	40.64	55.47	68.40	83.11	39.23	52.59	66.13	77.35	40.33	55.47	66.13	81.58
Daily gain g	27.9	35.6	31.5	39.9	27.4	35.3	36.6	36.1	25.6	31.6	31.6	35.5
	±	±	±	±	±	±	±	±	±	±	±	±
	2.14	1.83	1.22	4.87	1.50	1.87	1.47	4.92	1.59	1.52	1.52	2.76
Growth measure	1.46	1.55	2.17	2.08	1.43	1.49	1.80	2.14	1.58	1.75	2.09	2.29

Table 2

Average daily requirements, daily gain and growth measure among different groups of female chickens at the four periods

Group	I				II				III			
Period	1	2	3	4	1	2	3	4	1	2	3	4
Total Digestible Nutrients (T.D.N.) g	40.64	55.47	68.40	83.11	39.23	52.59	66.13	77.35	40.33	55.47	66.13	81.58
Daily gain g	23.7	29.5	29.3	30.5	24.7	30.5	31.1	24.2	22.1	25.8	28.5	25.7
	±	±	±	±	±	±	±	±	±	±	±	±
	1.71	1.88	2.33	2.73	1.59	1.72	2.12	3.19	1.82	2.15	2.32	3.17
Growth measure	0.90	1.53	1.60	1.93	1.07	1.13	1.48	1.56	1.08	1.51	1.21	1.32

Table 3

Average daily requirements, daily gain, growth measure and relative growth rate among different groups and sexes of chickens during the experimenal period (21—81 days)

Group	I		II		III	
	Male	Female	Male	Female	Male	Female
Total Digestible Nutrient (T.D.N.) g	61.91		58.83		60.88	
Daily gain g	33.73	28.25	33.85	27.63	31.10	25.53
Growth measure	1.84	2.19	1.74	2.13	1.96	2.38
Relative growth rate g	28.8	24.7	28.8	24.7	28.5	23.0

The average growth measure of the two sexes of Group II was lower than that of the other groups. Although the relative growth rate of the two sexes was the same in Group I and Group II being 28.8 g and 24.7 g for male and female, respectively, yet it was higher than that of Group III.

Discussion

From the present study it seems that the addition of DL-methionine to the deficient diet in sulphur amino acid to reach 0.90 per cent level improves the feed conversion and growth during the first, second and third period. In other words the most suitable daily requirement of sulphur amino acid for maximum feed conversion and maximum growth is 0.90 in the diet for both sexes of Hungarian white Leghorn growing chicks from 21 days old till 66 days of age, under Hungarian environmental conditions. This finding is in agreement with the recommended requirements of BRIGGS *et al.* (1942), GRAU-KAMEI (1950) and JUKES—STOKSTAD (1951).

ALMQUIST (1952) recommended 0.8 per cent sulphur amino acid in the diet, further noted that approximately 0.1 per cent more methionine was required in the diet for maximum efficiency of feed utilization than required for maximum growth.

Furthermore, the results showed that the quantitative requirement of the chick for sulphur amino acid during the period from 21—66 days of age was more than 0.62 and less than 1.2 per cent, approximately 0.9 per cent in the diet to support feed conversion and maximum growth for both sexes.

The results also indicated that added methionine at higher level (Group III) was of no value during the four periods. The average daily gain during the whole experimental period of Group III was lower than that of Group II being 2.75 g and 2.1 g less in case of male and female chicks respectively.

On the other hand, the growth measure was lower in Group II than in Group III, being 12.6 per cent less in male chicks.

Therefore, the excess of sulphur amino acids has a depressing effect on growth and efficiency of feed utilization. Similar effects were reported by NELSON *et al.* (1960) and BOLTON (1963).

Although the values of the growth measure and daily gain of Group II were better than both that of Group I and Group III during the first, second and third periods, these values — however — dropped in the last period. Such results indicated that the sulphur amino acid requirement might be more in young fowls having relatively more surface area per unit of body weight.

In this connection BOLTON (1963) reported that the methionine requirement for growth in the early growing period of chick was higher in relation to other amino acid than that of the older chick of the same breed, due to the development of the feather. This fact is based in part on the work of LEVIS *et al.* (1951), MENGE *et al.* (1953) and HILL (1953).

From the results procured in the present investigation good evidence was obtained of the beneficial effect on growth measure of fowls by the addition of DL-methionine during the four periods. In other words the addition of DL-methionine to the deficient diet at a rate of 0.9 per cent (Group II) improved the feed efficiency, even though there were no differences in daily gain (except the third period). Similar results were reported by ALMQUIST (1949), SAXENA—MC GINNIS (1952), RINGROSE—POTTER (1952), MATTERSON *et al.* (1953), REED *et al.* (1954) and NELSON *et al.* (1960). In this aspect SLINGER *et al.* (1953) mentioned that the addition of DL-methionine to the diets resulted in small but consistent improvement in feed efficiency but none of clearly defined influence on the average weights reached by the birds.

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THE RELATIONSHIP BETWEEN RICE EVAPOTRANSPIRATION AND DRY MATTER PRODUCTION

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The water use efficiency was increased at high plant density levels at 5 and 20 cms water depths. But the nitrogen levels and water depths did not influence water use efficiency. The yield/ET relationship gave a linear increase in water use efficiency with increasing yield.

Introduction

The relationship between the water use by rice and the harvestable yield is complex, but not irresolvable. The ET — yield data for a number of years will provide improved guide lines for management of limited water supplies and ultimately may permit reasonably accurate predictions of crop yield under given conditions of environment and water supply. Understanding these relationships will also clarify the nature of irrigation research data required in the future for further refinement of ET — yield functions.

The available data in rice are presented below: Kostyakov suggested a formula relating ET and yield as follows: $E = k \cdot t$, where E = ET in m^3/ha; k = water use coefficient in $m^3/1$ quintal grain; and t = planned production in quintals per ha.

In the Vietnam Democratic Republic, water use coefficients were 65 and 79 for summer and winter rice respectively.

Kostyakov reported from U.S.S.R., that substantially increased yields can be obtained by better fertilization and proper agrotechnical measures without increasing the total ET.

The relation between ET and yield of rice as reported by TSUTSUI (1966) in Japan is stated below:

Yield of rice. Kg per 10 acres	225—300	300—450	450—600
ET/yield	1.50	1.95	2.40

The above data bring out the fact that if the rice yield is to be increased, it is necessary to use a large amount of water.

Peterkung *et al.*(1965) reported that there was a positive correlation between transpiration rate and rice yield. Crops with higher yield consumed more water through plant transpiration. A normal crop of 4.5 tons per ha with an irrigation period of 100 days, consumed 6.3 mms per day of water by T. The above value declined to 1.4 mms when the yield decreased to 1 ton per ha. It increased to 10.5 mm when the yield reached 7.5 tons.

Anon (1965) and **Jana—Ghildyal** (1968) reported that more water use is not necessarily related to maximum dry matter production.

The above results are contradictory in nature and, therefore, no definite conclusions can be drawn. The water requirement and its reciprocal — water use efficiency — depend on the magnitude of two factors — ET and dry matter production. Unfortunately, data on these aspects of rice are lacking.

Material and Method

The study was conducted during the years 1968 and 1969 at the State Farm, Mezőtúr, a typical rice growing area of the Hungarian great plain. The elevation is 83 metres and it is situated 47° north, 20.31° east. The soil is solonchak. The average annual precipitation is 550 mm.

There were three factors, namely water depths (5 and 20 cm), N levels (40 and 80 kg/kh), and plant population (100 and 200 plants/sq.m), with all their combinations. Thus there were 8 treatments, replicated 4 times (Figs 1, 2).

The equipments used in the studies were galvanised iron tanks with an area of 2500 sq.cm. The tanks were embedded in the middle of the rice field, and all the treatments were replicated 4 times each. The ET was measured daily using the Héni-Tóth type gauge.

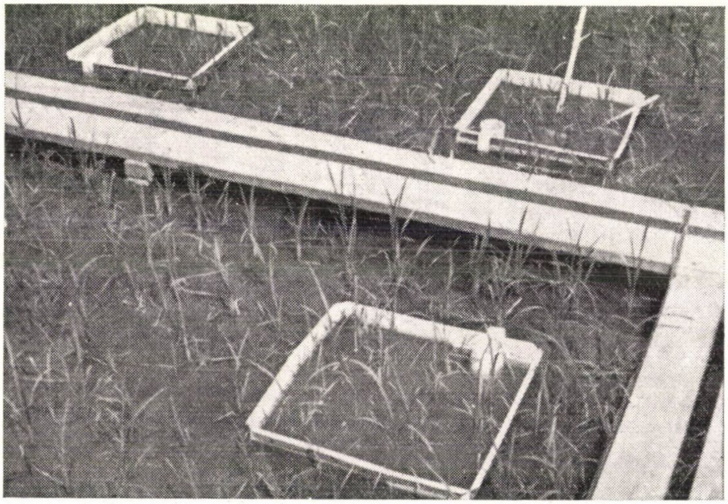

Fig. 1. A view of the tanks set in the rice field to measure evapotranspiration one month after sowing

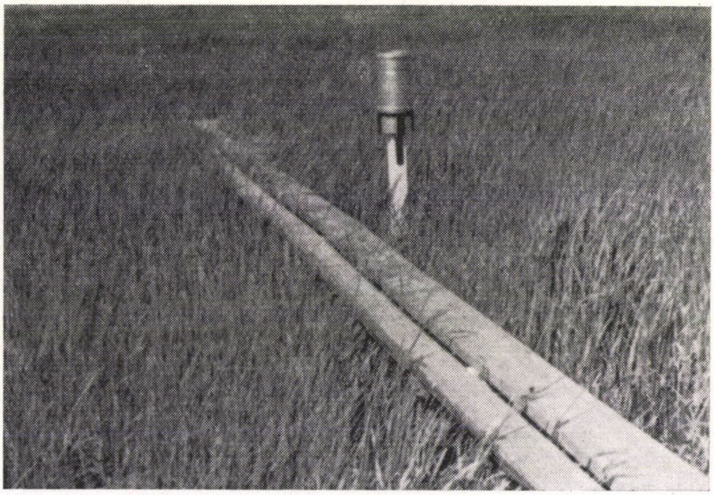

Fig. 2. Same at the active tillering stage. a) The relation between ET and yield of dry matter b) The relation between water use efficiency (Y/ET) and yield of dry matter

Results

The water use efficiency as influenced by nitrogen levels and plant density at two water depths are presented in Table 1.

It is observed that the quantity of water required to produce a unit quantity of dry matter generally decreases at a high plant density level (200 plants/sq. m.). Thus the water use efficiency increases. But high N level and water depth do not influence water use efficiency. These data illustrate the importance of adequate plant population, if maximum efficiency in water use

Table 1

The water use efficiency at different plant density

Year	plant density	5 cm					
		40 kg N/kh			80 kg N/kh		
		DM gm/tank	ET mm	ET/DM	DM gm/tank	ET mm	ET/DM
1968	100 plants/sq. m	168	476	2.8	157	493	3.1
	200 plants/sq. m	180	479	2.7	255	504	2.0
1969	100 plants/sq. m	160	558	3.5	160	569	3.6
	200 plants/sq. m	155	540	3.5	160	573	3.6

Year plant density		20 cms					
		40 kg N/kh			80 kg N/kh		
		DM gm/tank	ET mm	ET/DM	DM gm/tank	ET mm	ET/DM
1968	100 plants/sq. m	167	528	3.1	157	501	3.2
	200 plants/sq. m	240	536	2.2	219	518	2.4
1969	100 plants/sq. m.	158	609	3.9	138	584	4.2
	200 plants/sq. m	163	611	3.7	189	573	3.0

is to be realized. As plant density and N level increase, yield variability also increases, but there is little increase in the corresponding variability of ET. Thus, in this experiment N level and plant density are reflected in the crop yield, but do not cause a corresponding amount of variation in measured E. Differences in climatic conditions and low yield are responsible for the inefficiency of water use during 1966. It has been reported that increasing amount of yield of crop, produced by improving the crop environment, will not increase ET proportionately, provided that the crop is actively growing and is adequately supplied with soil moisture (STANBERRY 1957, STEED 1967).

The ET — Y relationships are shown in Figs 3a and b. This case gives a linear increase in water use efficiency with increasing yield.

ET in the rice field is mostly a physical phenomenon, provided that water is available for evaporation and the transpiration capacity of the plant is not limited by root damage or plant senescence. Yield, on the other hand, is a complex phenomenon depending to a large extent on soil variety and crop management practices, such as fertilization, plant spacing, and temperature that affect the production of leaf area and its assimilation rate in rice. Therefore it is reasonable to assume that ET is always at or near the radiation potential when the water is adequate, whereas the yield is not. So ET cannot increase faster than dry matter production.

The water use efficiency for a given crop and management practice, may be markedly affected by the climate. This is demonstrated by the data of 1968 and 1969 shown in Table 1. This was also observed in a general survey made by DEWIT in 1958. It may be emphasised again that, physically ET is almost solely a result of exposing a moist surface to a dry environment.

On the other hand, dry matter accumulation by plants is a much more involved process, even though certain ceonnections exist with ET notably through the climate, the ambient temperature and the stomatal mechanism.

Thus, it is possible to find greatly varying water use efficiencies in the growth of rice under different climates and cultural practices. The question of rice cultivation in areas where large amounts of sensible heat cause high rates of ET should be examined closely, since water use efficiencies may be comparatively low.

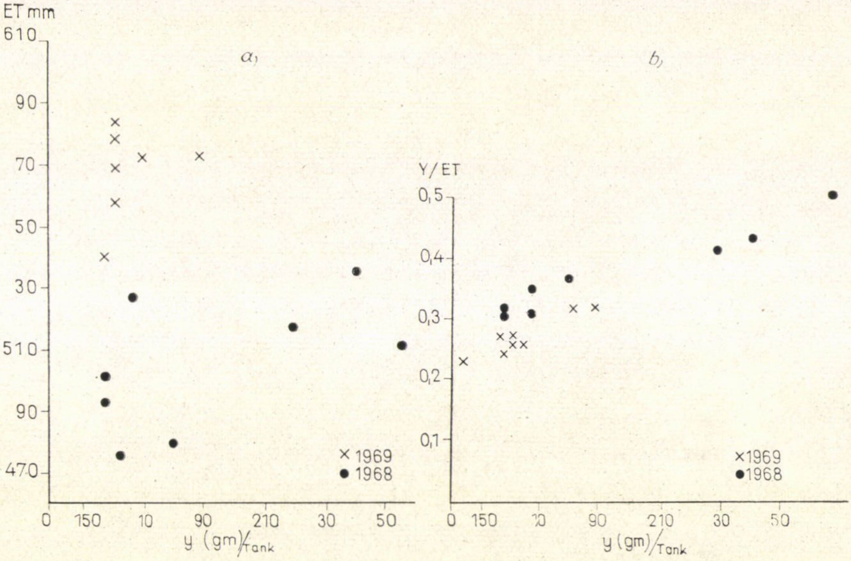

Fig. 3

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THE EFFECT OF COLCHICINE ON THE LEVEL OF LEAF PIGMENTS IN FLAX PLANTS

By

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The colchicine applicated on flax plants at their growing stage 50, 100 and 200 ppm as a fine spray, increased the level of chlorophyll-A, chlorophyll-B and xanthophyll. The content of carotene remained unaffected except the 200 ppm concentration. This positive effect of colchicine could be observed during the whole vegetation period in the relation of chlorophyll-A, and only till flowering in the relation of chlorophyll-B and xanthophyll. Treating the plants both at growing and flowering stages with colchicine influenced the level of chlorophyll-A and B, carotene and xanthophyll positively. This effect was very expressed at 100 and 200 ppm of colchicine and could be observed till maturity. The colchicine applicated on the plants three times during the vegetation period, namely at the growing, flowering and fruiting stages was ineffective in the relation of the pigments mentioned above, and the treatment after flowering gave no further increasing in the level of chlorophylls.

Introduction

The influence of colchicine on the polidia level of plants is well known, however, our knowledge about its primary physiological effect is incomplete.

Observed that 0.1 ppm colchicine increased the rate of respiration in *Rumex acetose*, and this extra energy was utilized for synthesis of organic compounds. Higher doses than 0.1 ppm retarded the process mentioned above. Studying the effect of colchicine on the rate of growth of *Cunninghamella elegans* Naguib (1963, 1964, 1967, 1968), Naguib *et al.* (1964, 1966a, 1966b) demonstrated that 10 ppm colchicine enhanced phosphorus uptake, inhibited peptone and protein synthesis. High doses of colchicine considerably decreased the rate of nitrate and protein uptake.

Fahmy *et al.* (1970) observed that the colchicine did not affect the content of monosaccharides in the soaked kenaf germs. Lower doses of colchicine increased both the sucrose content and total nitrogen level.

The physiological effect of colchicine on flax has not been studied. The results obtained in connection with the influence of colchicine treatment on the level of some leaf pigments are summarized in this paper.

Material and Method

The experiments were carried out in pots with flax (Giza 6) under green-house conditions at *Giza Research Station* during the years 1969—70. Each pot contained 11 kg of loam, 5.5. g superphosphate, 3.0 g of ammonium nitrate, 2.5 g of potassium chloride and 30—35 flax plants. The experiment started on the 27th November and the yield was gathered on 7th May each year

250 ml of 50, 100 and 200 ppm colchicine (Sandoz) solution was used as fine spray material. The plants were treated two or three times at growing, growing and flowering, growing-flowering and fruiting stages.

The plants were analysed four times during the vegetation period. The determination of pigments was carried out chromatographically.

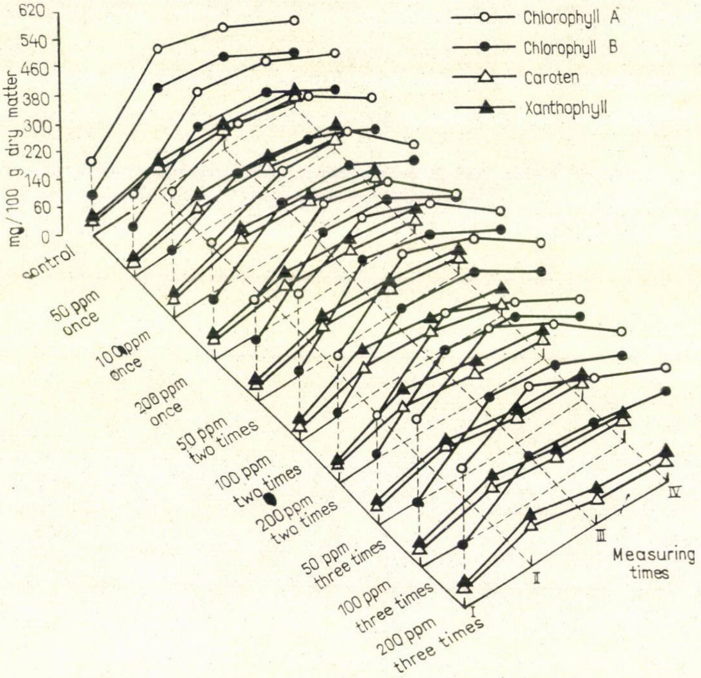

Fig. 1. The effect of colchicine on the pigment content of the flax leaves

Results

In Figure 1 it can be observed that the colchicine applicated at the growing stage significantly increased both the chlorophyll-A and B content in the leaf. Increasing of the chlorophyll-A content could be observed during the whole vegetation period and was the most expressed at the concentration level of 100 and 200 ppm colchicine.

When the plants were treated two times, both at growing and flowering stage, with 100 and 200 ppm colchicine, 20—30% increase could be observed in both chlorophyll contents, and this positive effect could be recognized till fruiting or maturity.

The plants treated at growing, flowering and fruiting stages contained more chlorophyll-A and B than the control. The third treatment, performed during the fruiting stage did not cause the further increase of the green pigment level.

In the same figure it can be seen that the treatment at the growing stage with different concentrations of colchicine caused 20—36% increase in the xanthophyll-level of the leaves and this effect could be recognized till flowering. The carotene content in these treatments was not affected except of 200 ppm.

Colchicine applicated two times both at growing and flowering stages increased the carotene and xanthophyll level. The phenomenon was expressed and well defineable till fruiting and maturity in the relation of xanthophyll.

Further treatment at fruiting stage had no effect on carotene, however, the amount of xanthophyll changed as that of the variant mentioned above.

Conclusion

The results of the experiments showed that colchicine applied once at growing stage increased the level of photosynthetic pigments in the flax plants. This positive effect of colchicine treatment can be recognized only till flowering. Spraying with colchicine solution at fruiting stage had no further effect on pigment content. The effect of colchicine on the change of chlorophyll-A and B level was different.

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EFFECT OF ZINC ON NODULATION AND YIELD OF SOYBEAN (GLYCINE MAX.)

By

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It was found in general that there was a reduction in grain yield with the application of zinc. Higher levels of zinc also suppressed nodulation, leghaemoglobin synthesis and bacteroid formation.

Introduction

Micronutrient research work in India as well as abroad has shown that there are wide genotypic variations in the susceptibility of crop plants to micronutrient deficiencies (AGARWALA *et al.* 1969, BROWN 1963, MASSEY—LOEFFEL 1966, MUNNS *et al.* 1963, POLSON 1968, SAXENA—SINGH 1970, VOSE 1963). Most of the soils particulaly in North India which are under the cultivation of rice and wheat crops are reported to be zinc deficient. There is a paucity of information on the relative requirement of the soybean crop to zinc in our country. Also no work has been reported on the effect of zinc on the nodulation and leghaemoglobin synthesis of soybean.

Material and Method

A pot culture experiment was conducted with four replications during the spring season of 1971. The soil under precent investigation was of a silty clay loam type. The experiment was laid out in a simple randomized block design with 3 varieties of spring soybean (merritt, adelphia and traverse) and 3 levels of zinc (0, 5 ppm and 10 ppm) applied in the form of zinc sulphate (chemically pure) to the soil at the time of sowing. Phosphate and potash were applied at the rates of 100 kg P_2O_5/ha and 40 kg K_2O/ha in the form of single superphosphate and potassium sulphate respectively. Seeds were inoculated with a Peat-based multi-strained *Rhizobium japonicum* culture of nitrogen and were sown immediately. Dry matter yield, number and dry weight of nodules per plant basis were recorded when the crop was 54 days old. The leghaemoglobin content was estimated by following the method given by BERGERSON (1961) and the total number of bacteroids were also determined after the method described by BERGERSON (1960). The total content of zinc was determined by the dithizone method as outlined in the procedures edited by BLACK (1965) and its uptake was calculated. All these observations were taken when the crop attained 54 days of age. Grain yield per plant was determined at the time of harvest and was statistically analysed (YATES 1937). Correlations were made wherever possible by following the method given by SNEDECOR (1946).

Results

It was found, in general, that the higher levels of zinc increased the uptake of zinc. On the contrary, there was a reduction in the number of nodules, even dry weight of nodules, leghaemoglobin synthesis and bacteroid contents with an increase in the levels of zinc in all the varieties. The reduction was in the order 0, 5, 10 ppm zinc respectively. However, in case of merritt, the number of nodules and their oven dry weight increased up to a level of 5 ppm zinc application and thereafter it showed a sudden decline at 10 ppm level. Among the varieties tested adelphia showed maximum number and dry weight of nodules per plant, leghaemoglobin synthesis and bacteroid content. The only other treatment where this was noticed was in the one, where no zinc application was given (Table 1).

Table 1

Effect of different varieties of soybean and levels of zinc on nodulation and zinc uptake by soybean

Variety	Level of zinc (ppm)	No. of nodules/plant	Oven dry weight of nodules/plant (g)	Leghaemoglobin content (μg)	No. of bacteroids (X 10^4)	Zinc uptake (ppm)
Merritt	0	8.5	0.360	35.10	1780	100
	5	16.0	0.472	31.72	1653	132
	10	3.0	0.188	29.10	1214	125
Adelphia	0	33.0	0.959	284.21	3411	84
	5	12.0	0.373	30.50	1624	137
	10	16.0	0.282	40.00	3057	262
Traverse	0	9.0	0.101	20.55	421	120
	5	4.0	0.118	20.25	418	160
	10	4.0	0.120	25.27	520	184

Significant difference was noticed among varieties and levels of zinc as far as the yield was concerned. Among varieties, adelphia yielded significantly more than the merritt and traverse varieties (Table 2). Considerable reduction was noticed due to the application of zinc. It was found that there was no significant difference between 0 and 5 ppm levels of zinc, but application of zinc at 10 ppm level brought a significantly lower yield than that of the 0 and 5 ppm levels.

The interaction between varieties and levels of zinc also revealed certain interesting results as far as grain yield was concerned (Table 3). Zinc application at levels of 5 and 10 ppm brought significant reduction in yield compared to the control in the case of the merritt variety. Whereas in the case of the

Table 2

Effect of different varieties and levels of zinc on the grain yield of soybean

Treatment	Grain yield/plant (g)
Varieties:	
Merritt	25.47
Adelphia	32.23
Traverse	21.07
Levels of zinc:	
0 ppm	28.68
5 ppm	28.22
10 ppm	21.87
S.Em.	±0.8198
C.D. at 5%	1.6822
C.D. at 1%	2.2708

Table 3

Interaction between varieties and levels of zinc application on the grain yield of soybean

Variety	Grain yield/plant (g) Level of zinc application		
	0 ppm	5 ppm	10 ppm
Merritt	10.80	7.14	7.54
Adelphia	11.51	12.56	8.16
Traverse	6.38	8.52	6.17
S.Em.		±1.3421	
C.D. at 5%		2.7530	
C.D. at 1%		—	

adelphia variety 10 ppm zinc application yielded significantly less than that of the 0 and 5 ppm levels, respectively. No significant difference was noticed in the case of the traverse variety with zinc application.

Discussion

Application of zinc at a higher level in general, had an adverse effect on grain yield in the varieties tested in the present investigation. This was due to a reduction in the number of nodules and the dry weight of nodules per plant.

Soybean plants nodulated by an ineffective strain showed the lowest dry matter yield, nitrogen content and plant height (MARTINEZ *et al.* 1970, TILAK 1971). Application of zinc may probably be connected with the ineffectiveness of *Rhizobium japonicum*, thereby retarding nodulation.

Nodules that are effective in nitrogen fixation contain leghaemoglobin. It is present within the membrane envelope surrounding the bacteroids and is not free in the plant or bacteroid cytoplasm (XOCHUM, 1964). Thus we may presume that bacteroids produce this pigment. In the present investigation it was found that the control (without zinc application) had more leghaemoglobin and bacteroid contents than those treated with zinc. So zinc may, perhaps, be responsible for inhibiting bacteroid formation and thereby repressing leghaemoglobin synthesis. A negative correlation was seen between leghaemoglobin and bacteroid contents ($r = 0.6957$). This, in turn, caused a reduction in the number of nodules.

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VARIA

BROOM CORN "MEZŐKOVÁCSHÁZI"

Taxonomic place: *Sorgum dochna* (Forsk.) Snowden var. *technicum* (Koern.) Snowden

Origin: selected from a landrace population at Mezőkovácsháza

Beginning of breeding: 1947, Medgyesegyháza

Breeder: Pál Bacsa dr. and Pál Tóth, Kiszombor

State qualification: Provisionally certified improved variety, 1957; State certified variety, 1959

General characterization: a high (difficult to mechanize) broom corn with dark brown spikelets, providing excellent industrial raw material

Morphological description:

Root system: penetrates deep into the soil (150—200 cm)

Shoot system: bushy, with intensive tillering

Stem: may reach 3—3.5 m, with about eight nodes; of light green colour, resistant to lodging

Foliage: leaf blade is light green, leaf sheath dark green; length of blade 60—70 cm, width 8—9 cm; ligula dark green (NAGY 1971)

Panicle: generally 40—60 cm long with 52—60 branches which are dark green and excellently fine when flowering. According to many years data some 55 per cent of the harvested crop consists of first class broomcorn beard. The colour of the spikelets is dark brown (from the colour of the glumes)

Caryopsis: about 4—5 mm long, remains covered by glumes; the glume-covered grain is pear-shaped, the edges of the glumes are thickly hairy, their surface is shiny. The grain is brown

Biological characters:

Germination: optimum at a soil temperature of about 11°C

Vegetative period: 155—160 days

Development: rapid and vigorous; panicles appear at the beginning of August and ripen toward the end of September (KAPÁS *et al.* 1965)

Resistance to disease: sufficient

Farm technology requirements:

Seeding: at the end of April; in the author's experiments sowing on 28th April provided the best industrial raw material (NAGY 1971). Spacing: $40-50\times 20$ cm; quantity of seed grain required: 9—12 kg/cad.yoke (1 cad.yoke = 5754.56 m^2)

Soil requirements: in fertile soils gives high yields (KAPÁS *et al.* 1965)

Productivity: awn: 6.7 q/cad.yoke; seed grain production: 13—15 q/cad.yoke (BACSA 1957). The quality of the awn depends on the weather conditions. Best quality panicles are obtained with sowing at the end of April

Area of cultivation: best grown on the area of Békés county; here it has a closed growing district (KAPÁS *et al.* 1965)

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STUDIES ON THE EGYPTIAN BROAD BEAN SEEDS.
I. CHEMICAL CONSTITUENTS OF THE BROAD BEAN SEEDS

Broad bean *(Vicia faba)* is one of the legumes commonly grown and used in large quantities. It plays an increasingly important role in the diets of the people in Egypt, and is commonly eaten as the first meal of the day.

The starch content of the seeds was found to be 37.08%, giving two fractions, amylose (15%), and amylopectin (85%). No free reducing sugars were found in the water extracts. GHALI (1955) found that the sucrose content was 1.98% in the cotyledons and 1.74% in the whole seeds. KAWAMURA—NAKAMURA (1954), KAWAMURA (1954) in a study on the carbohydrates of the broad bean seeds, found that the total carbohydrates were 46—49%, the starch was 39.9%, while the reducing sugars, and non-reducing sugars were 1.1% and 4% respectively. He reported that acid hydrolysis and paper chromatographic analysis for the polysaccharides, showed the presence of fructose, glucose, xylose and rhamnose. Fucose was also reported as a constituent of the hemicellulose of the broad bean seeds (KAWAMURA 1959).

The amino acid composition of the broad bean seeds was estimated using microbiological assays and chromatographic analysis. Eighteen amino acids were detected and quantitatively estimated (GHALI 1955, KHAN—BAKER 1957, MASSOUD 1958, PION *et al.* 1963, INAMDER—SOHONIE 1960).

The present paper deals with the component and nutritional quality of the broad bean seeds (Giza I and Giza II). More interest was given to the carbohydrate and amino acid pattern of the two varieties in a trial to select the varieties yielding protein quality superior to that of those which are at present consumed in Egypt.

Sampling. One kg of *Vicia faba* of each of the two varieties Giza I and Giza II which were obtained from the plant breeding department, Ministry of Agriculture, were finely ground. Samples from the ground materials were analysed. Other samples were prepared by getting out the rinds from the cotyledons and the two components were finely ground.

Methods of analysis. The samples were analysed for moisture, nitrogen, fat, crude fiber, ash and iron using the usual standard methods (A.O.A.C. 1965).

Estimation of calcium and phosphorus was as described by STUFFINS (1967).

Identification and determination of sugars. Sugars were extracted with 80% neutral ethanol, concentrated under vacuum and fractionated chromatographically on a paper chromatogram using a mixture of pyridine, ethyl acetate, water 1 : 2.5 : 3.5 v/v (upper layer) for 18 hrs at 20°C. The descending technique was carried out and the sugars were located using aniline oxalate as developing reagent. Reducing sugars were determined by the method of SOMOGYI (1945).

Identification and determination of polysaccharides. The polysaccharides present in both the flesh of the rinds and the cotyledons were hydrolysed with sulphuric acid solution (1 N) for 16 hrs on a boiling water bath. After neutralization and concentration under vacuum the aliquots were fractionated chromatographically. The starch component was determined by the CLEGG method (1956).

Determination of the amino acids. Samples were hydrolysed with 6 N HCl for 22—24 hrs at 110°C according to the KHAN—BAKER method (1955). HCl was evaporated off under vacuum to dryness and the residues were dissolved in a certain volume of 10% isopropyl alcohol. The portions were spotted on the Whatman No. 1 filter paper and two dimensional ascending chromatograms were prepared using the buffered method given by LEVEY—CHUNG (1953), using n-butanol–acetic acid–water 12 : 3 : 5 (v/v) as the first solvent for 24 hrs, followed by m-cresol–phenol mixture (2 : 1) in borate buffer pH 8.3 for 26 hrs at 20°C. Development took place using the ninhydrin reagent 0.2% in ethanol and acetic acid, and the isatin reagent (0.2% in acetone) for the proline.

The located amino acids were eluted in 75% aqueous ethanol containing copper nitrate, and the acetone–water mixture (2 : 1 v/v) for the proline. The concentrations of each individual amino acid were determined colourimetrically at 510 $m\mu$ and proline at 570 $m\mu$.

Estimation of cysteine and methionine (JAMALIAN—PELLET 1968). A quantity of the sample was weighed in a conical flask and then placed in an ice bath and cooled to 0°C. The cold performic acid reagent was added to the sample and oxidation of the sample was allowed to continue at 0°C for 16 hrs. The oxidizing solution was evaporated under vacuum at 30-35°C, after which it was allowed to hydrolyse in the HCl solution (6 N) for 24 hrs. The hydrochloric acid was removed by repeated concentration in a rotatory evaporator and the hydrolysate was finally made up to a certain volume by isopropyl alcohol 10% and the cysteine and methionine were determined by the paper chromatography technique as mentioned before.

Determination of tryptophan (LAMBORD—DCLANGE 1965). The tryptophan content was determined colourimetrically after alkali hydrolysis with barium hydroxide solution (14%) for 22 hours. All the amino acids were calculated from standard curves.

The chemical analysis of the two varieties of broad bean are summarized in Table 1.

From the results given in Table 1 there was no marked difference in the chemical constituents of the two varieties. Meanwhile they are good sources of protein, phosphorus and calcium and are somewhat deficient in iron.

Table 1

Chemical constituents of the broad bean seeds (Vicia faba) (dry basis)

Chemical constituents	Giza I variety	Giza II variety
Moisture %	10.35	10.30
Total carbohydrates %	46.42	45.83
Total protein %	28.10	28.62
Ether extract %	1.12	1.10
Crude fiber %	5.90	6.08
Ash %	4.22	4.35
Phosphorus mg/100 gm.	898.00	925.00
Iron mg/100 gm.	4.20	4.33
Calcium mg/100 gm.	213.30	219.70

Table 2

Carbohydrate content of the Egyptian broad bean seeds (dry basis)

Carbohydrate constituent	Giza I	Giza II
Reducing sugars	7.20	7.50
Non-reducing sugars	1.76	1.83
Total sugars	8.96	9.33
Starch	37.46	36.50

The carbohydrate contents of the broad bean seeds reached to about 46.4%. The starch content represents the major reserve polysaccharide (37.46%) as shown in Table 2, while the reducing sugars ranged between 7.20—7.50%, and the non reducing sugars from 1.7—1.8%. The paper chromatographic analysis revealed the presence of galactose and sucrose in the aqueous alcohol extracts of the cotyledons, and acid hydrolysis for the polysaccharides showed the presence of glucose and arabinose. Xylose and glucose were detected in the alcohol extracts of the rinds while the polysaccharides contained xylose, glucose, galactose and arabinose. These results confirm the previous findings by Ghali (1955) and Kawamura—Nakamura (1954), Kawamura (1954) but the presence of galactose in the aqueous alcohol extract of the cotyledons was not previously detected and this may be due to the varietal differences. The determination of the protein contents of the seeds in the two varieties Giza I and Giza II showed a high protein content (28.10 and 28.62%).

The acid hydrolysis for the proteins and the determination of the amino acids by the chromatographic analysis, showed the presence of sixteen amino acids. As shown in Table 3, it is quite evident that the seeds contained most of the essential amino acids required for human consumption: glutamic acid, aspartic acid, sarine, glycine, phenylalanine, valine, leucine and isoleucine, arginine, lysine, and valine were present in relatively larger amounts. Most increases were attributable to glutamic acid, aspartic acid, leucine, isoleucine, and lysine. The determination indicates that the two varieties are poor sources of the sulphur containing

Table 3

Amino acid composition of Egyptian broad bean seeds (dry wt. basis)

Amino acids	mg/100 gm		Mg./g. N	
	Giza I	Giza II	Giza I	Giza II
Aspartic acid	2125	2080	530	507
Glutamic acid	4186	4254	1026	1023
Serine	1325	1341	331	327
Glycine	1305	1318	326	321
Threonine	965	943	237	227
Alanine	900	916	221	220
Lysine	1533	1645	376	395
Arginine	1415	1434	347	345
Tyrosine	1001	1121	245	270
Valine	1370	1335	336	321
Leucine + Isoleucine	3308	3328	811	800
Phenylalanine	1112	1148	273	276
Tryptophane	272	270	67	65
Cystine	218	226	53	54
Methionine	159	170	39	41

amino acids such as methionine and cystine and are somewhat deficient in the tryptophan. There were no differences in the amounts of the amino acids with the exception of lysine and tyrosine which were relatively higher in the Giza II than the Giza I variety which was rich in the values of valine, leucines and threonine.

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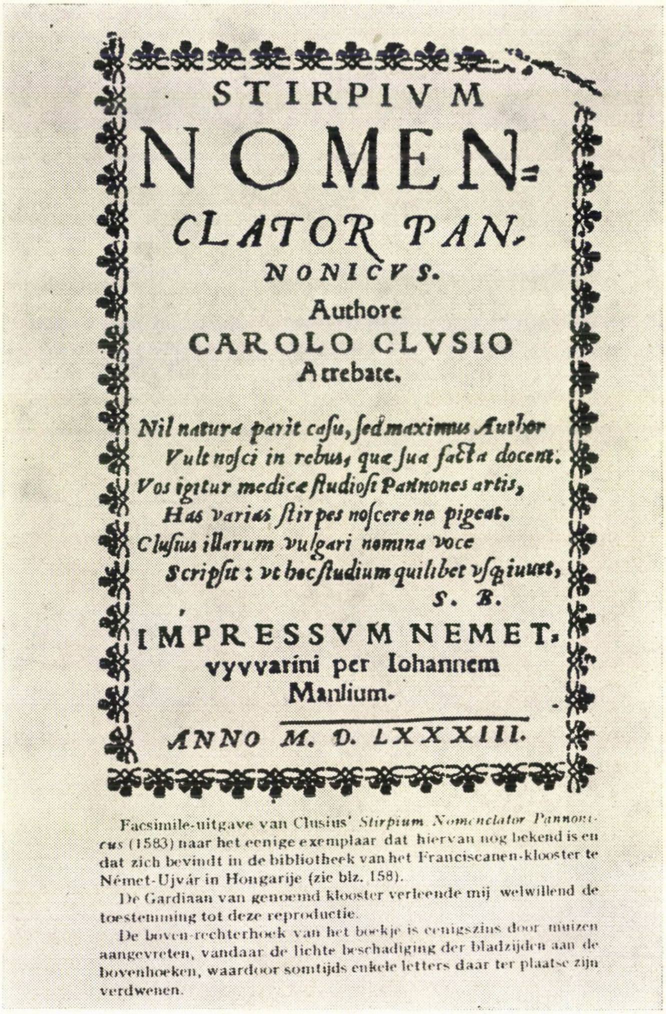
CLUSIUS—BEYTHE: STIRPIUM NOMENCLATOR PANNONICUS



STIRPIVM
NOMEN-
CLATOR PAN-
NONICVS.
Authore
CAROLO CLVSIO
Atrebate.

Nil naturæ parit caſu, ſed maximus Author
Vult noſci in rebus, quæ ſua facta docent.
Vos igitur medicæ ſtudioſi Pannones artis,
Has varias ſtirpes noſcere ne pigeat.
Cluſius illarum vulgari nomina voce
Scripſit: vt hoc ſtudium quilibet vſq; iuuet,
S. B.

IMPRESSVM NEMET-
vyvvarini per Iohannem
Manlium.

ANNO M. D. LXXXIII.

Facsimile-uitgave van Clusius' *Stirpium Nomenclator Pannonicus* (1583) naar het eenige exemplaar dat hiervan nog bekend is en dat zich bevindt in de bibliotheek van het Franciscanen-klooster te Német-Ujvár in Hongarije (zie blz. 158).

De Gardiaan van genoemd klooster verleende mij welwillend de toestemming tot deze reproductie.

De boven-rechterhoek van het boekje is eenigszins door muizen aangevreten, vandaar de lichte beschadiging der bladzijden aan de bovenhoeken, waardoor somtijds enkele letters daar ter plaatse zijn verdwenen.

Fig. 1. Publication of the title page of the Stirpium Nomenclator Pannonicus faximile (Hungarian edition)

The *Stirpium Nomenclator Pannonicus*, the catalogue of the Pannonian plants is a small Latin-Hungarian onomasticon printed in eights. It was first published in Hungary, in 1583, then in Belgium, in 1584. Although no indication of the growing sites of plants is found in the book, it must be considered one of the first attempts to describe the Hungarian flora. After Melius' Herbarium published in 1578 this was the second botanical booklet written in Hungarian.

The Hungarian edition was published in 1583 at Németújvár, on the estate of Boldizsár Batthyány (1543—1590), a Hungarian aristocrat of humanist education, in János

Manlius' printing house, in an extension of 16 pages. This edition, however, was almost completely lost due partly to the small number of copies published, partly to the permanent war situation existing at that time in Hungary. The only copy left was found by László Fejérpataky in the library of the Franciscan cloister at Németújvár, 300 years after the publication, in 1883. Of this copy a facsimile has been made which is kept in the National Széchényi Library at number RMK. II. 175.

The Belgian edition was published in 1584 in Antwerp, in Plantin's printing house though not in a separate volume but as a part of Clusius' "*Rariorum aliquot Stirpium, per Pannoniam, Austriam et vicinas quasdem Provincias obseruatarum Historia*", which from a Hungarian point of view is Clusius' most important, what is more: epoch-making work. The latter work ran into several editions, each differing from the others; in one thing, however, they agree: the Nomenclator Pannonicus is contained in all of them (Fig. 2). The Nomenclator Pannonicus should be regarded as the complement of Clusius' great work mentioned above. Namely, the plants listed in it were collected by Clusius too. However, in his great work mentioned above he described only the ones occurring less frequently. More common plants are listed only in this catalogue.

When the two editions of the Nomenclator Pannonicus are compared evidence is given to settle a much discussed problem: to make sure the person of the author; further, an example is presented of the importance of international scientific cooperation in the 16th century.

Even the front-pages of the two editions are different. On the front-page of the Hungarian edition we find the name of Clusius as author: "*Authore Carolo Clusio Atrebate*". On the other hand, a hexastichon written by István Beythe in praise of Clusius is printed on this front-page:

"*Nil natura parit casu, sed maximus Author*
Vult nosci in rebus, quae sua facta docent.
Vos igitur medicae studiosi Pannones artis,
Has varias stripes noscere ne pigeat.
Clusius illarum vulgari nomina voce
Scripsit: ut hoc studium quilibet usque iuuet.

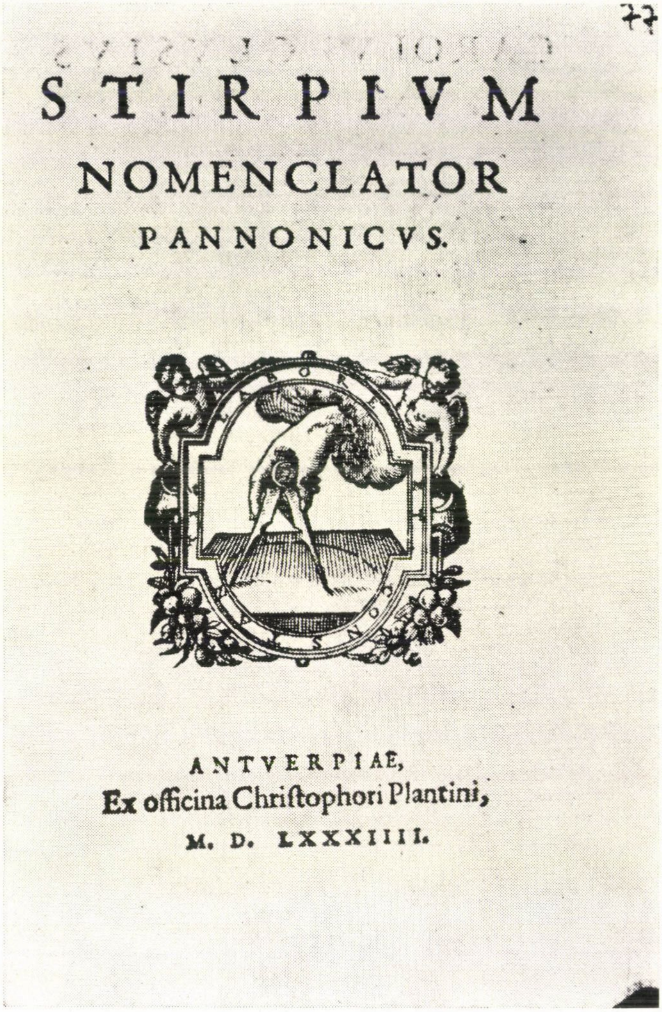
S. B."

"Nothing is born from nature, but everything from the supreme Creator who wants to teach you through His deeds. Thus, you who in Pannonia study the art of medicine do not refuse the knowledge of these plants. Clusius gave them popular names so that anybody can make use of this book."

(The Hungarian translation is a measured poem written by János Bódás.)

Until the Hungarian edition was found in 1883 at Németújvár some scientists had thought that the Nomenclator Pannonicus was Beythe's work. After the Hungarian scientists foreign authors (e.g. Pritzel: Thesaurus Litt. Bot. 1851, p. 47, and 1872, p. 64) too attributed this work to Beythe. There were even some of them who accused Clusius of having kept Beythe's name back in the Antwerp edition. After the discovery of the Németújvár edition the scientists did justice to Clusius pointing out that the Nomenclator Pannonicus is the result of a joint work done by Clusius and Beythe. This statement could be made with great certainty since that front-page of the Németújvár edition — prepared for the press undoubtedly by Beythe — proved unambiguously the authorship as well as the useful and imperishable work of Clusius.

The preface was published in the two editions almost with the same text. Clusius speaks in it to the botanists of Hungary, asking them to extend the Nomenclator Panno-



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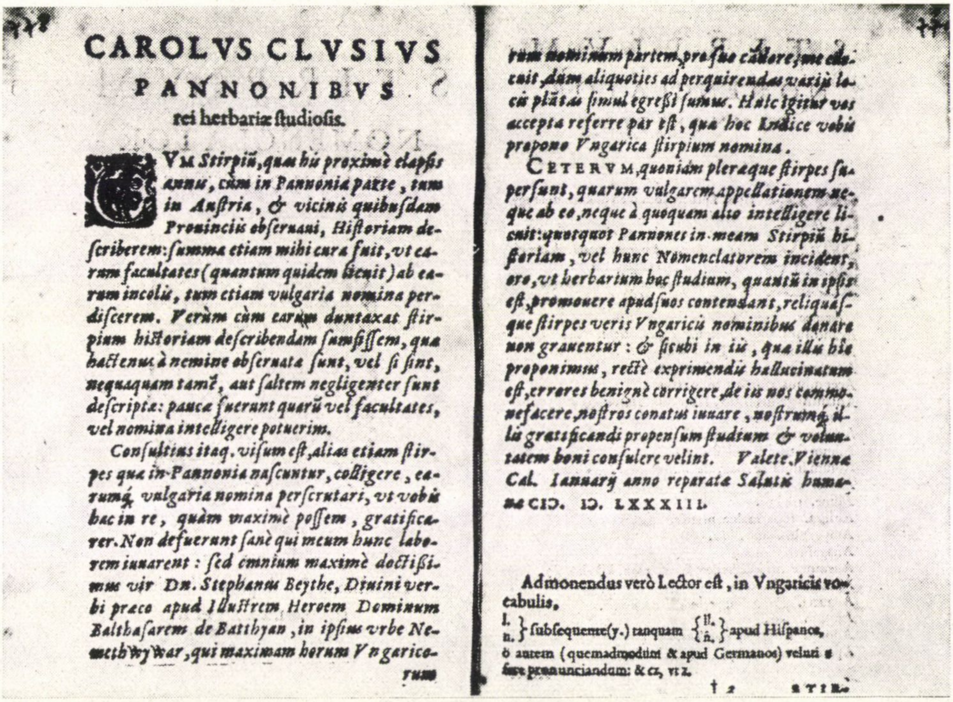
STIRPIVM
NOMENCLATOR
PANNONICVS.

ANTVERPIAE,
Ex officina Chriſtophori Plantini,
M. D. LXXXIIII.

Fig. 2. Publication of the title page of the Stirpium Nomenclator Pannonicus faximile (Belgian edition)

nicus and give names to the other Hungarian plants too. In this preface dated from Vienna in January 1583 Clusius writes, among others the following: "... it seemed more advisable to collect other plants growing in Pannonia too, and find out their popular names in order to please you as much as possible. There were, of course, many who helped me in this work, but the greatest assistance of all was given by István Beythe, the scholarly preacher of the Word of God at Németújvár, on the estate of the honourable Boldizsár Batthyány, who taught me most of these Hungarian names when on several occasions we went together to search for plants at different places." (Fig. 3.)

Thus, while on the front-page of the Németújvár edition it was Beythe who pointed to Clusius' merits, in the preface Clusius thanked István Beythe, the "preacher of great



CAROLVS CLVSIVS
PANNONIBVS
rei herbariæ ſtudioſis.

CVm Stirpiũ, quas his proximè elapſis annis, cùm in Pannonia parte, tum in Auſtria, & vicinis quibuſdam Prouinciis obſeruaui, Hiſtoriam deſcriberem: ſumma etiam mihi cura fuit, vt earum facultates (quantum quidem licuit) ab earum incolis, tum etiam vulgaria nomina perdiſcerem. Verùm cùm earum duntaxat ſtirpium hiſtoriam deſcribendam ſumpſiſſem, quæ hactenus à nemine obſeruatæ ſunt, vel ſi ſint, nequaquam tamẽ, aut ſaltem negligenter ſunt deſcriptæ: paucæ fuerunt quarũ vel facultates, vel nomina intelligere potuerim.

Conſultius itaq. viſum eſt, alias etiam ſtirpes quæ in Pannonia naſcuntur, colligere, earumq́ vulgaria nomina perſcrutari, vt vobis hac in re, quàm maximè poſſem, gratificarer. Non defuerunt ſanè qui meum hunc laborem iuuarent: ſed omnium maximè doctiſſimus vir Dn. Stephanus Beythe, Diuini verbi præco apud Illuſtrem Heroem Dominum Balthaſarem de Batthyan, in ipſius vrbe Nemethwywar, qui maximam horum Vngaricorum

rum

rum nominum partem, pro ſuo candore, me edocuit, dum aliquoties ad perquirendas variis locis plãtas ſimul egreſſi ſumus. Hæc igitur vos accepta referre par eſt, quæ hoc Indice vobis propono Vngarica ſtirpium nomina.

CETERVM, quoniam pleraque ſtirpes ſuperſunt, quarum vulgarem appellationem neque ab eo, neque à quoquam alio intelligere licuit: quotquot Pannones in meam Stirpiũ hiſtoriam, vel hunc Nomenclatorem incident, oro, vt herbarium hoc ſtudium, quantũ in ipſis eſt, promouere apud ſuos contendant, reliquaſque ſtirpes veris Vngaricis nominibus donare non grauentur: & ſicubi in iis, quæ illis hic proponimus, rectè exprimendis hallucinatum eſt, errores benignè corrigere, de iis nos commonefacere, noſtros conatus iuuare, noſtrumq́ illis gratificandi propenſum ſtudium & voluntatem boni conſulere velint. Valete. Viennæ Cal. Ianuarij anno reparatæ Salutis humanæ CIↃ. IↃ. LXXXIII.

Admonendus verò Lector eſt, in Vngaricis vocabulis,
l. n. } ſubſequente (y.) tanquam { ll. ñ. } apud Hiſpanos,
ò autem (quemadmodum & apud Germanos) veluti [illegible] fere pronunciandum: & cz, vt z.

† 2 STIR-

Fig. 3. Introduction to the Hungarian edition

learning", for his assistance. It was in that way that they set an imperishable memory for each other and became the ideals of scientists even in the 20th century.

The preface is followed by the list of plants. The first plant in both editions is "Abies, spruce, of which the leaves grow in one direction". And in both editions the list is closed by "*Zea, spelta, spelt*". But this does not mean that the two editions are identical in every respect. Apart from minor differences mainly related with the printing technics, the following substantial differences are found between the two editions. (When comparing the two editions we give the text in a faithful copy leaving the misprints in it.)

Németújvár edition 1583	*Antwerp edition 1584*
Adianthum, ruta muraria, Keu fali ruta. (Rue)	*Adianthi genus illud, Ruta muraria vulgo nuncupatum.* Keu fali ruta. (Rue)
Anemone siluestris, kekerchen, feyer veragu. (anemone, white flowered)	*Anemone sil.* kekerchen, feyer veragu, kikeleti fiu. (anemone, white flowered)
Armoracia, Torma. *Nasturcium vocant vulgo Vngari.* (Horse radish)	*Armoracia, siue Raphanus sil. quorundam Gallorum Cran.* Torma. *vulgo Nasturtium Latine Vngari vocant.* (Horse radish)
Artemisia tenuifolia, sepreu fiu, *hoc est scoparum herba, quod ex ea scopae vulgo fiant.*	*Artemisia tenuifolia.* sepreu fiu h.e. *scoparum herba: quod ex ea scopae apud Vngaros fieri soleant. Ob quem etiam vsum Austriaci besemkraut appellant.*

Arundo nad. *Atriplex* östör. (Orache) Bugla, töuisk ali seb fiu. *Chamaemelum*, siue Camomilla, zék fiu. (Chamomile) *Cornus*, som fa. (Bunch-berry)	
Corylus, *nux auellana*, Monyaro fa, *eius iulus*, monyaro barkocza. (Hazel)	*Corylus, nux auellana siue pontica.* monyaró fa. (Hazel) *Coryli iulus, siue flos.* monyaro barkocza.
Cotoneum, bis alma. (Quince) *Cuminum* Kömeny (Cumin) *Dracunculus* kygyo trank. *Eruum*, mohar köles. (Hungarian millet) *Eupatorium, vide Agrimonia.* *Ficus*, fighe (Fig) *Flos*, verag. (Flower)	
Frangula, *alnus nigra*, bidös fa.	*Frangula Matthioli*, *Alnus nigra Dodonaei.* bidös fa. *Fungus nuncupatus Crepitus lupinus.* pöfeteg. (Puff-ball)
Heliotropium, napra nézö fiu. *Labrusca*, vad zölö. (Wild vine)	
Liliasphodelus flore luteo, zöld Liliom, *crotae apud quos albo flore inuenirit in Pannonicis obseruationibus scripsi*, Liuolye, Illyan zuet. *Karcissus*, Saffran zinö verag. özzel. (Narcissus) *Nepeta*, matra fiu.	*Liliasphodelus luteo flore.* zöld liliom.
Primula veris, kasa verag. tauazi elsö verag. (Primrose)	*Primula veris.* kasa verag, tauazi elsö verag, h.e. *milij contriti flos (praefertim pallida ἄχαυλος* et *simplici praedita flore, passim in Vngaria et frequentissime nascens) quod milij contrici*, Kasa *ab Vngaris appellati, colorem referat.*
Pyrola, teuisk ali fiuechke.	
	Quercus robur. Kemeni cherfa. (Robur)
Santonicum barany iröm. *Seriphium*, tengöri iröm. *Siligo*, öregbed ros.	

As you can see the Németújvár edition includes 20 plants which are missing in the Antwerp edition. On the other hand, there are four plants in the Antwerp edition which are not found in the Németújvár edition. This edition gives, further, a more detailed description of the plants while the Németújvár edition does this only in the cases of Liliasphodelus flore luteo and *Pseudodamasonium* (in the other edition: *Pseudodamasionum*). All this proves — in our opinion — that the Antwerp edition was prepared for the press by Clusius while the Németújvár edition by Beythe. Beythe, otherwise, must have been a connoisseur of plants, and it is regrettable that he left no such work behind as the one Melius presented us with. Summing up: in the Németújvár edition the number of plants listed is 346, while in the Antwerp edition 330.

The importance of this list is increased by the fact that it includes 235 plants which are not found in Clusius' other works. If we do not count the cultivated and foreign plants there are still 145 plants left which increase the number of Hungarian plants mentioned by Clusius, says Endre Gombocz.

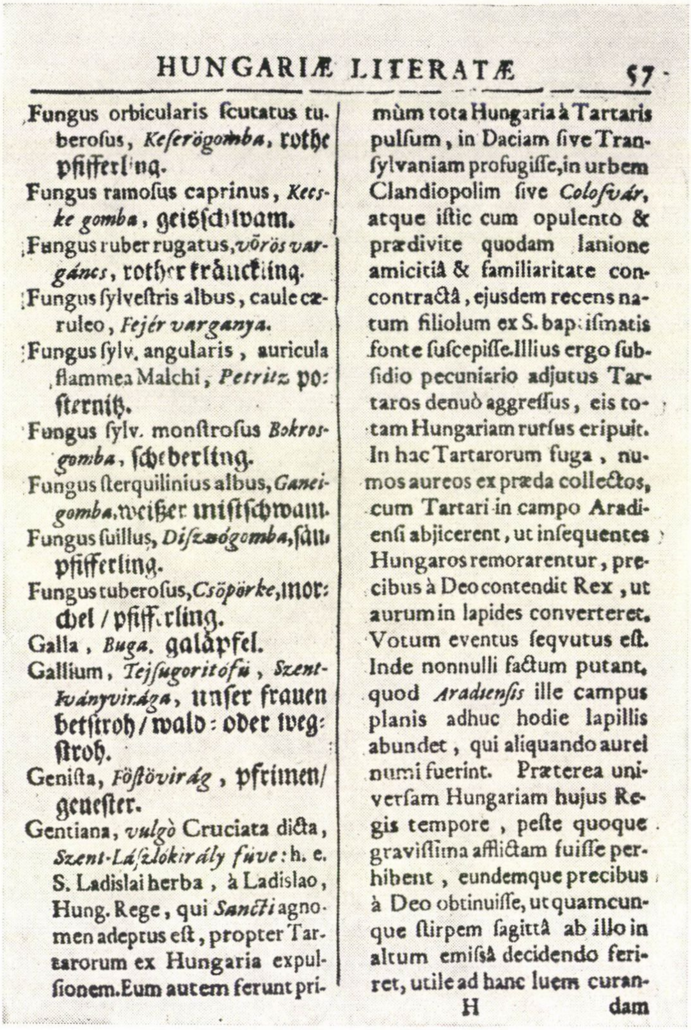
It was not only from a botanical point of view that the Nomenclator Pannonicus was important, but also as a study on the Hungarian language, as it is a rich source of Hungarian plant names. The possibility of the complete loss of the booklet was all the more distressing. For this very reason David Czvittinger (1676—1743) preserved it in full for us on pages 51—66 of his great work "*Specimen Hungariae Literatae*" published in Frankfurt and Leipzig in 1711. (The preface was included, however, only in part.) His aim — as he himself said — was partly to save it from complete destruction, partly to encourage those who like botany in Hungary to extend this list.

Czvittinger not only corrected the Hungarian names which had been printed wrongly — in and old-fashioned way —, but also extended the list by writing the German name after the name of each plant through which the bilingual Nomenclator Pannonicus became trilingual, on one hand; and by listing more than 30 such plants as not contained in either of the two editions, on the other. They are: *Acacia*, *Acetosa*, *Amaracus*, Bacheku (radix Indiae Bagolygomba, bachkres), *Cinamonum* (sic), 17 species of *Fungus*, *Malus aurantia*, *Malus citri*, *Olea*, *Tabacum*, etc.

It is obvious again that Czvittinger did not know about the Németújvár edition. It is not mentioned in the introduction either, and — above all — of the 20 plants which are found exclusively in the Németújvár edition only 3 are contained in Czvittinger's book which, however, he published beyond a doubt with the names he added to the list: *Atriplex*, *Ficus*, *Narcissus*. On the other hand, he did not include e.g. *Hieracium* and *Quercus latifolia* either, although they are found in the Antwerp edition too.

The Nomenclator Pannonicus is thus known today in 3 variations. By studying and comparing them our botanists and linguists may find further treasures.

The Nomenclator Pannonicus has another remarkable feature which must be mentioned here. Namely, while listing the plants it preserved for us two old legends related to two grasses. After listing the "*Gentiana*, *quae vulgo cruciata dicitur*, zent Lazlo király fiue, *hoc est*, *S. Ladislai Regis herba*" it tells the following legend in Latin: "László, the first Hungarian king with this name, who later was called saint for expelling the Tatars from Hungary is said to have been chased earlier by the Tatars throughout Hungary and fled to Dacia i.e. Transylvania, to the town of Claudius, better known as Kolozsvár. Here he made friends with a very rich and wealthy butcher and became related to him by marriage. With the help of this butcher who covered the costs of war László at last attacked the Tatars and took the whole Hungary back from them. While chasing the Tatars — when they dispersed the plundered golden coins in order to delay the Hungarians in chasing them — he beseeched God to change the coins into stones. His prayer was granted. The flat stones, the fields near Arad are full of, are said to have been golden coins earlier. It is said that during the reign of this king the



HUNGARIÆ LITERATÆ 57

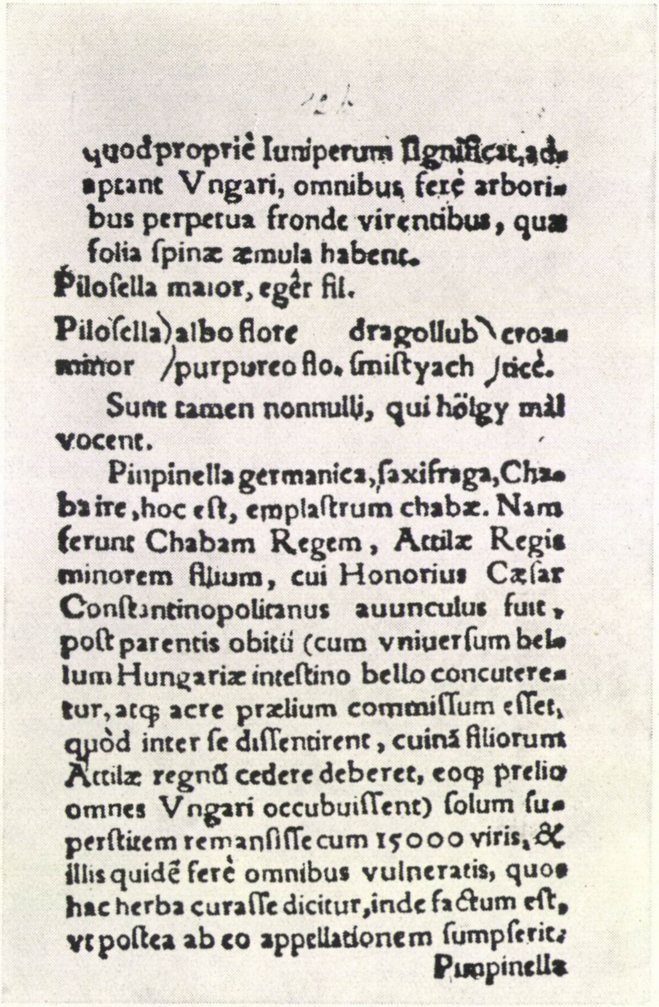
Fungus orbicularis ſcutatus tuberoſus, *Keſerögomba*, rothe pfifferling.
Fungus ramoſus caprinus, *Kecske gomba*, geißſchwam.
Fungus ruber rugatus, *vörös vargáncs*, rother kräuckling.
Fungus ſylveſtris albus, caule cæruleo, *Fejér varganya.*
Fungus ſylv. angularis, auricula flammea Malchi, *Petritz* poſternitz.
Fungus ſylv. monſtroſus *Bokrosgomba*, ſcheberling.
Fungus ſterquilinius albus, *Ganeigomba*, weißer miſtſchwam.
Fungus ſuillus, *Diſznógomba*, ſau pfifferling.
Fungus tuberoſus, *Csöpörke*, morchel / pfifferling.
Galla, *Buga.* galäpfel.
Gallium, *Tejſugoritófü*, *Szent-Ivány virága*, unſer frauen betſtroh / wald: oder weg: ſtroh.
Genista, *Föſtövirág*, pfrimen / geneſter.
Gentiana, *vulgò* Cruciata dicta, *Szent-Láſzlókirály füve*: h. e. S. Ladislai herba, à Ladislao, Hung. Rege, qui *Sancti* agnomen adeptus eſt, propter Tartarorum ex Hungaria expulſionem. Eum autem ferunt primùm tota Hungaria à Tartaris pulſum, in Daciam ſive Tranſylvaniam profugiſſe, in urbem Clandiopolim ſive *Coloſvár*, atque iſtic cum opulento & prædivite quodam lanione amicitiâ & familiaritate concontractâ, ejusdem recens natum filiolum ex S. baptiſmatis fonte ſuſcepiſſe. Illius ergo ſubſidio pecuniario adjutus Tartaros denuò aggreſſus, eis totam Hungariam rurſus eripuit. In hac Tartarorum fuga, numos aureos ex præda collectos, cum Tartari in campo Aradienſi abjicerent, ut inſequentes Hungaros remorarentur, precibus à Deo contendit Rex, ut aurum in lapides converteret. Votum eventus ſeqvutus eſt. Inde nonnulli factum putant, quod *Aradienſis* ille campus planis adhuc hodie lapillis abundet, qui aliquando aurei numi fuerint. Præterea univerſam Hungariam hujus Regis tempore, peſte quoque graviſſima afflictam fuiſſe perhibent, eundemque precibus à Deo obtinuiſſe, ut quamcunque ſtirpem ſagittâ ab illo in altum emiſsâ decidendo feriret, utile ad hanc luem curan-

H dam

Fig. 4. A page from the Hungarian edition

whole of Hungary was stricken with plague, but as an answer to his prayers to God the plant hit by his arrow was capable to cure this disease. The arrow is said to have hit 'cruciata' with which the king then cured his subjects from the infection of the plague." (After the Németújvár edition, Fig. 4.)

Another legend has been preserved too in the Nomenclator Pannonicus, that related to the pimpinella. "*Pimpinella germanica, Saxifraga* (kőtörőfű) Csaba's balm or Csaba's plaster. (Chabaeemplastrum.) It is said, namely, that Prince Csaba, King Attila's youngest son born from the daughter of Caesar Honorius fought his brothers Ellák and Dinció for the inheritance of the kingdom after his father's death. They were defeated and slaughtered by the Ostrogoths who formed an alliance with Ardaric, king of the Gepids, only Csaba sur-



quod propriè Iuniperum ſignificat, adaptant Vngari, omnibus ferè arboribus perpetua fronde virentibus, quæ folia ſpinæ æmula habent.

Piloſella maior, egêr fil.

Piloſella) albo flore dragollub\ croa-
minor / purpureo flo. ſmiſtyach / ticè.

Sunt tamen nonnulli, qui hölgy màl vocent.

Pinpinella germanica, ſaxifraga, Chabaire, hoc eſt, emplaſtrum chabæ. Nam ferunt Chabam Regem, Attilæ Regis minorem filium, cui Honorius Cæſar Conſtantinopolitanus auunculus fuit, poſt parentis obitū (cum vniuerſum bellum Hungariæ inteſtino bello concuteretur, atq; acre prælium commiſſum eſſet, quòd inter ſe diſſentirent, cuinā filiorum Attilæ regnū cederet deberet, eoq; prelio omnes Vngari occubuiſſent) ſolum ſuperſtitem remanſiſſe cum 15000 viris, & illis quidē ferè omnibus vulneratis, quos hac herba curaſſe dicitur, inde factum eſt, vt poſtea ab eo appellationem ſumpſerit.

Pimpinella

Fig. 5. The legend connected with Pimpinella germanica

vived unhurt the battle with 15.000 of his men, who were, however, wounded in one or another part of their bodies. But the above mentioned grass placed by pure chance on their wounds cured them, and that is why it was called later "Csaba's plaster", or "Csaba's balm". (After Czvittinger, Fig. 5.)

The Nomenclator Pannonicus did a great service merely by recording these two legends. The legend of St. László's grass was written up by János Arany, the greatest Hungarian epic poet (1817—1882) in a poem consisting of three parts and 53 four-line stanzas. The same poet intended to write about the Csaba's balm in the Csaba-trilogy, but only a sketch was completed. As to the Csaba's balm (pimpinella) László Arany (son of János Arany) wrote in a note the following: "See Ipolyi: Magyar Mythologia (Hungarian Mythology) 160. The

Hungarian name of this grass of miraculous power, and the legend related with it are found — apart from the living popular tradition — in a 16th century herbal too."

A last question must by all means touched upon: namely, the question of what is to be understood by the name of Pannonia. It was a much discussed problem over a long period what Clusius had understood by Pannonia. However, Károly Flatt pointed out with locality data as a confirmation that by Clusius' Pannonia a part of Hungary is to be understood. And while it is beyond question that the lines bordering Clusius' collecting area are not as distinct as the political borders, it is still quite certain that when in other works he mentions "in utraque Pannonia" he thinks of the present Transdanubia and Croatia (Yugoslavia) and not of Lower Austria or Styria which he generally mentions separately.

As you have seen in the preface of the Nomenclator Pannonicus Clusius called upon the botanists of Hungary to extend the Nomenclator Pannonicus and give Hungarian names to the other plants grown in Hungary too. But how long this must have been waited for is shown by the fact that after Melius and Beythe József Csapó's (1734—1799) work: "Uj füves és virágos magyar kert" (Hungarian grasses and flowers), 1775 was the first botanical work written in Hungarian which tried to give a botanical knowledge to those unfamiliar with the Latin language.

P. Hargita

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CHENOPODIUM POLYSPERMUM AND DATURA INOXIA AS NEW TEST PLANTS FOR TWO STRAINS OF ALFALFA MOSAIC VIRUS

The host range of alfalfa mosaic virus (R/1 : 1,3/18 : U/U : S/Ap, AMV) is known to be very wide (reviewed by Thornberry 1966, Quantz 1968, Crill *et al.* 1970) and it is still developing continuously (Beczner—Schmelzer 1972a, b, Schmelzer *et al.* 1972). Plant

Table 1

Reactions of Chenopodium and Datura species against AMV

Family and species	Reaction		References
	AMV-L	AMV-S	
Chenopodiaceae			
*Chenopodium album***	L S	L S	1
*C. amaranticolor***	L S	L S	1
C. ambrosioides	L	L	Schmelzer 1962—63
C. botrys	L S	—	Horváth—Beczner 1968
C. capitatum	(L) S	S	Horváth—Beczner 1968 Verhoyen 1964
C. ficifolium	+	—	Schmelzer—Schmidt 1968
C. foetidum	L S	L S	Horváth—Beczner 1968 Schmelzer 1962/63
C. glaucum	L S	L S	Beczner—Schmelzer 1972
C. murale	L	L	Schmelzer 1962—63 Beczner 1967
C. polyspermum	L S	L S	new host
*C. quinoa***	L S	L S	1
C. rubrum	L S	L S	Beczner—Schmelzer 1972
C. vulvaria	L S	L S	Lovisolo 1962
Solanaceae			
Datura inoxia	(L) S	(L) S	new host
D. metel	L S	L S	Crill *et. al.* 1970
D. meteloides	S	S	Lovisolo 1962
*D. stramonium*** var. *stramonium*	L S	(L) S	1
var. *inermis*	L S	(L) S	
D. tatula	L S	(L) S	Nagaich—Giri 1968

L = local, S = systemic symptoms, / / = mild or occasional, + = susceptible, — = not tested, ** = natural hosts of AMV, 1 = very frequently used host (e.g. reviewed by Thornberry 1966, Hull 1969, Crill *et al.* 1970)

species belonging to the families Chenopodiaceae and Solanaceae, are especially susceptible to AMV. They are important from the point of view of identification, too.

Of the genus *Chenopodium* which belongs to the family *Chenopodiaceae* the following species were studied with respect to their role as indicator or test plants: *Chenopodium album*, *C. amaranticolor*, *C. ambrosioides*, *C. botrys*, *C. capitatum*, *C. ficifolium*, *C. foetidum*, *C. glaucum*, *C. murale*, *C. polyspermum*, *C. quinoa*, *C. rubrum* and *C. vulvaria*.

Of the genus Datura which belongs to the family Solanaceae the following species were

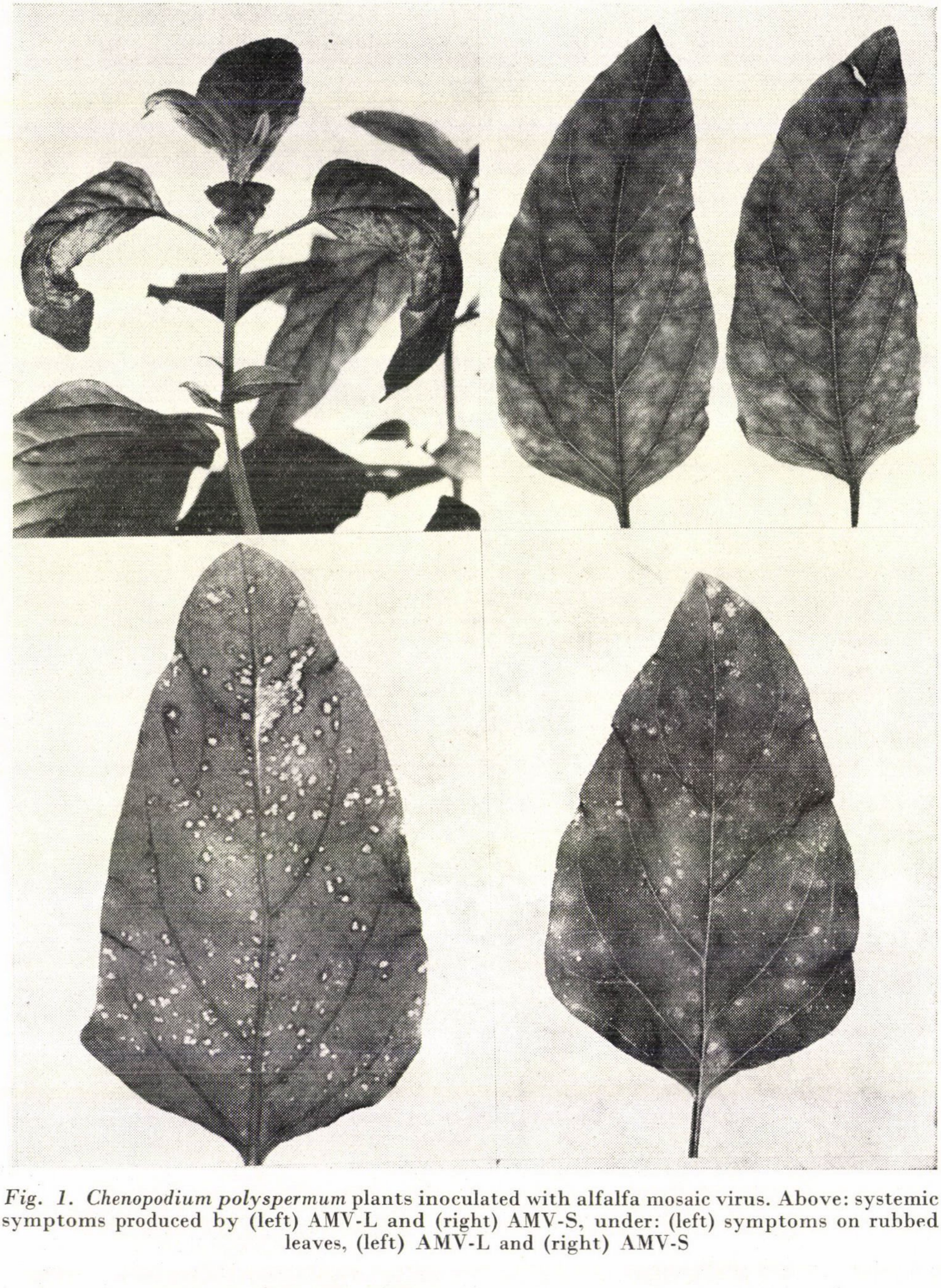

Fig. 1. Chenopodium polyspermum plants inoculated with alfalfa mosaic virus. Above: systemic symptoms produced by (left) AMV-L and (right) AMV-S, under: (left) symptoms on rubbed leaves, (left) AMV-L and (right) AMV-S

Fig. 2. Datura inoxia plants inoculated with alfalfa mosaic virus. Above: (left) yellow mosaic, (right) chlorotic spots on rubbed leaves and vein clearing appeared first after inoculation, under: (right) mosaic and leaf deformation in chronic stage, (left) D. metel showed systemic symptom

studied: *D. inoxia*, *D. metel*, *D. meteloides*, *D. stramonium* var. *stramonium*, *D. stramonium* var. *inermis* and *D. tatula*.

The aim of the present study was to test whether or not *Chenopodium* and *Datura* species, not studied so far in this respect could be used as test plants for AMV and to reinvestigate the reactions of the species mentioned in Table 1 to two different types of AMV strains.

For these experiments two strains (cf. Beczner 1967, 1972) of AMV were used. The methods of preparing and using the inocula have already been described in previous papers (Beczner 1967, Beczner—Schmelzer 1972).

Results are given in Table 1. In general, AMV-L isolate produced more severe symptoms on all test plants used, than those produced by AMV-S. *Chenopodium* species, except *C. polyspermum* are well known hosts for AMV-L and AMV-S, giving the same reactions with the two virus strains. AMV-S caused fewer local lesions and milder systemic (mosaic of leaf deformation) symptoms. A new experimental host, *C. polyspermum* reacted locally in 3—5 days after inoculation and systematically within further 2 or 4 days. The local lesions were surrounded with a brown necrotic zone in the case of AMV-L infection, while AMV-S caused chlorotic local lesions (Fig. 1).

The diagnostic value of *C. polyspermum* is especially important because of the production of pronounced and countable local lesions.

It was found that *Datura* species reacted with local and systemic symptoms to the two types of AMV strains. Upon infection with AMV-L the mosaic and deformation symptoms became especially severe. *Datura inoxia* proved to be a new host of AMV. It produced chlorotic (occasionally necrotic) local lesions on rubbed leaf and vein clearing and deformation of following leaves (Fig. 2). This new host will improve an artificial system for differentiating various AMV isolates into severe, mild and weak groups.

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L. Beczner

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FRUIT SET PROMOTED BY CHEMICAL INDUCTION IN PÁNDY SOUR CHERRY

The low fertility and unreliable productivity of the Pándy sour cherry is generally known. It is one of the important objectives of sour cherry production to increase the reliability of production in the completely self-sterile Pándy sour cherry.

The productivity of varieties can be increased partly by breeding methods, partly by suitable pollen varieties, the right choice of their proportions and optimum arrangement within a plantation. The reliability of production, on the other hand, is promoted — besides the growing site and disease resistance — by the natural and induced parthenocarpy.

In stone fruits natural parthenocarpy does not occur, therefore from the point of view of increasing the reliability of production fruit set induced by chemicals is of great importance.

Investigations aimed at increasing the fruit set of Pándy sour cherry were carried out in 1968 and 1969 with trees of medium trunk height grown on Prunus mahaleb rootstocks, transplanted at the Érd-Elvira station of the Horticultural Research Institute at a spacing of 8×8 m. The present paper gives an account of the preliminary results.

The ability to become fertile through free pollination is highly varying in the Pándy sour cherry clons, the investigations were, therefore, performed with grafts of clonal scion. The treatments of the experiment were set on five trees of the early flowering clon of Pándy number 48. In the years of the experiment the weather at the time of flowering varied greatly, as shown by the differences in fertility and productivity between the years.

In 1968 flowering began on 9 April which was followed by heavy snow. Air temperature fell considerably, and frost set in at dawn (—4.5°C). The cold weather inhibited the flowering and prevented the fruit set for two weeks.

In 1969, as a result of a rapid rise in the temperature, the flowering of Pándy — 48 suddenly set in on 19 April. Owing to the high protracted temperature (+25°C) the duration of flowering was very short.

During the experiments the following observations were made in the Pándy No. 48 clon: *a)* The ability to become fertile through free pollination. All open flowers were counted at the four cardinal points of the five trees marked out per treatment, in definite parts at a medium height in the crown and on this basis the percentage proportion of flowers in the variety collection of which fruit developed under natural conditions was determined. *b)* The effects of various chemical treatments on fruit set and fruit quality. At the phenophase of bud bursting the flowers were isolated with parchment paper bags. Chemical treatments were applied on two occasions: in full blossom (in the isolators 70—80 per cent of the flowers were open), and 14 days later. The various concentration solutions were applied to the open

Table 1

Effect of treatments with various chemicals on fruit set and fruit quality in the clon Pándy No. 48

(Érd-Elvira, 1968, 1969)

Treatment			Year	Number of flowers treated	Fruit set at ripening time %	Average fruit weight g
Chemical	Concentration (ppm)	time				
Control	—	—	1968	15.678	3.2	6.4
			1969	17.512	10.5	5.2
GA_3	100	full blossom	1968	2.354	0.0	—
			1969	5.862	0.0	—
GA_3	250	full blossom	1968	2.011	0.0	—
			1969	4.350	0.0	—
GA_3	500	full blossom	1968	1.873	0.0	—
			1969	6.210	0.0	—
GA_3	100	full blossom, then two weeks later repeatedly	1968	2.481	0.0	—
			1969	4.392	0.0	—
GA_3	250	full blossom, then two weeks later repeatedly	1968	3.117	0.0	
			1969	3.028	0.0	—
GA_3	500	full blossom, then two weeks later repeatedly	1968	2.973	0.0	—
			1969	4.521	0.0	—
2,4-D	50	full blossom	1968	3.183	3.5	2.7
			1969	4.215	4.1	2.5
2,4,-D	50	full blossom, then two weeks later repeatedly	1968	3.121	3.5	2.3
			1969	3.016	5.2	2.8
2,4,5-T	30	full blossom	1968	3.850	10.5	2.0
			1969	2.975	9.2	2.1
2,4,5-T	30	full blossom, then two weeks later repeatedly	1968	3.010	15.1	2.4
			1969	3.527	17.3	2.3
GA_3+2,4-D	250+50	full blossom	1968	3.270	8.3	2.9
			1969	4.013	9.2	3.1
GA_3+2,4-D	250+50	full blossom, then two weeks later repeatedly	1968	4.879	15.7	3.0
			1969	4.218	12.1	2.8
GA_3+2,4,5-T	250+30	full blossom	1968	2.997	38.6	2.6
			1969	3.526	27.5	2.9
GA_3+2,4,5-T	250+30	full blossom, then two weeks later repeatedly	1968	3.107	53.1	2.3
			1969	3.301	48.7	2.4

flowers by painting. The following chemicals were included in the experiments: gibberellic acid (GA_3), dichlorophenoxy-acetic acid (2.4-D), 2,4,5-trichlorophenoxy-acetic acid (2,4,5-T); Tween-20 (polyoxy-ethylene-sorbitane monolaurate) was used at 0.05 per cent as wetting agent. The isolators were removed immediately after flower shedding.

Fruit set was assessed at the time of ripening and expressed by the percentage proportion of fruits set to the total number of flowers treated. To characterize the effect of the treatments on the quality of the fruit we determined the average fruit weight. The effect of chemical treatments of various concentration applied at different times on fruit set and fruit quality is shown in Table 1.

According to the results of the investigations, the highest extent of parthenocarpic fruit set — as compared to the control, set through cross pollination — was induced by a combined treatment with GA_3 250 ppm + 2,4,5-T 30 ppm applied in full blossom and repeated two weeks later (in the free pollinated control fruit set at the time of ripening was 3.2—10.5 per cent, while in the above treatment it ranged between 48.7 and 53.1 per cent). The two synthetic auxins included in the experiments had a favourable effect on fruit set both when applied by themselves and when combined with GA_3. GA_3 in itself was not found efficient in any of the treatments.

The chemically induced fruits of the Pándy sour cherry were all parthenocarpic. Fruit size showed a high heterogeneity at the time of ripening. The economically valuable parthenocarpic fruits had an average weight of 2.0—3.1 g, while the weight of fruits set by cross pollination was 5.2—6.4 g on an average.

Under the influence of certain treatments the time of ripening also changed. The most remarkable change was observed with the GA_3 + 2,4,5-T treatments: fruits ripened 1.5—2 weeks later than those of the control.

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PLASMON—GENOME CONDITIONED POLLEN LETHALITY IN EU-OENOTHERAE

In Oenothera of the sub-genus *Eu-Oenothera* there can be found two pollen-patterns: in the homozygote species a homogeneous pattern is visible with only full, normal grains, while the heterogeneous species reveal three grain-classes with a differently pronounced lethality. We examined the determinism of this lethality. In an earlier work the effect of a lethal gene was excluded (Linder—Jean 1968). From pollen examinations of bastards between homozygote and heterozygote species a plasmatic condition of pollen lethality can be presumed: always according to the harmonic or disharmonic combination of the genome and of the plasmon in the microspore, the pollen grain is viable or lethal. In this sense — for the gamethophyte — we have taken the genetic system which was developed by Stubbe (1959, 1963) into consideration as an explanation for the leaf-chimeras in the Oenothera bastards. Recent works of Göpel (1970) and Arnold (1970) similarly lead, on another experimental road, to the plasmatic condition of pollen lethality.

The pollen patterns of hybrids between homozygote species should provide an enlightment, since no lethality is present in the parents, while it may occur in the bastard-progenies. *A* — the two employed homozygote species:

The homozygote species consist of different genetic types:

(1) the nature-spontaneous homozygotes, the area of which extends on the western border of the U.S.A. (CLELAND 1962):

Oenothera hookeri Torrey et Gray (forma Johanssen)
Oenothera franciscana Bartl.

Both species have the genome (plastom-combination AA)I.

(2) the translocation-mutated homozygotes. They derive from heterozygotes through translocation. This occurs more frequently in species which are in possession of the velans complex, as e.g. in *Oenothera conferta* Renner. The newly originated complex has an almost velans-similar genotype. Two types are distinguished:

(a) the semi-homozygotes or semi-mutants (according to RENNER 1941). They derive from *Oe. coronifera* and *Oe. conferta* and are morphologically characterized by the following features: clumsy floral buds, sepals which strike the eye by their dark-red stripes; thence the rubrisepala denomination given by Renner to these mutants.

— *Oenothera coronifera* forma *rubrisepala* (abbreviated: *Oe. coronifera rubs.*). The diakinase-view shows a 4-ring and 5 bivalents (4, 5×2). The plant is isogametic and transfers the subvelans and velans complexes. The pollen is divided into three classes (48 per cent active, 37 per cent inactive and 15 per cent empty grains). The genome/plastom combination is AA/II. We received these translocation-mutants from the Renner Collection, Munich.

— *Oenothera conferta* forma *rubrisepala* (abbreviated: *Oe. conferta rubs.*). The diakenase-view similarly shows a 4-ring and 5 bivalents. The plant is distinguished from the abovenamed by an almost homogeneous pollen-population (17 per cent smaller grains, and 3 per cent empty). This form has turned up in our breeding, from *Oe. conferta* of the Renner collection.

(b) The full-homozygotes or full-mutants. They show 7 bivalents in the diakinase and are total homozygotes. The pollen is homogeneous. The following species have been employed:

— *Oenothera blandina* de Vries. This mutant originates from *Oe. lamarckiana;* its genome/plastom-combination is AA/III.

— *Oenothera purpurata* Klebahn. It is described by RUDLOFF (1929) in its place of discovery on the Lüneburger lowland plain. Its origin is unknown. The genome/plastom-combination is AA/II.

These two species are identical with the natural homozygotes. We are bringing them together under the denomination "full-homozygotes", in order to distinguish them from the semi-homozygotes.

These six different homozygotes have been employed by us to perform crossings between (1) full-homozygotes between themselves, (2) between full- and semi-homozygotes.

B — The pollen pattern of bastards between full-homozygotes:

Oenothera hookeri, *Oe. franciscana*, *Oe. blandina* and *Oe. purpurata* are in the possession of the same geno-type A, but of different plastoms (I, II, III). In the present experiment the interaction between a single genom and different plastoms will therefore be compared.

(1) Results of the experiments.

For each bastard at least 3 stamina from 3 plants were examined. All examinations of the same bastard always presented the same pollen-pattern. The results are indicated in Table 1.

From the reciprocal crossings between *Oe. hookeri*, *Oe. blandina* and *Oe. franciscana* a homogeneous pollen pattern is shown by the bastards. However, with *Oe. purpurata* effected crossings resulted in plants in which there occurred pollen-lethality: the pollen pattern is heterogenous, the grains are unevenly large, the percentage of empty grains fluctuates between 7 and 30 per cent.

Bastards of reciprocal crossings between *Oe. hookeri* and *Oe. purpurata* are remarkable. If *hookeri* is the mother, the pollen pattern is heterogeneous, but when purpurata is the mother, the lethality is intensified and the grains can be divided into three classes: 68 per cent, active, 8 per cent inactive and 24 per cent are empty.

(2) Discussion.

Oenothera purpurata carries the lethality by its plasmon into the pollen and thereby produces a pollen-pattern in the bastard which resembles that of a heterozygote *Oenothera*. It is thus established, that the *hookeri* × *purpurata* bastard contains the maternal *hookeri*-plasmon, but also elements of the *purpurata*-plasmon, which are transmitted through the pollen-tube. After the meiosis the *purpurata*-plasmon in the microspore can be left out through somatical splitting. In this case a normal pollen-grain comes into being. When, however, *purpurata*-plasmon remains in the microspore, then the pollen-grain regresses. As a final result, we have a heterogeneous pollen-pattern.

In the reciprocal crossing *purpurata* × *hookeri* the plasmon is of the *purpurata*-type, to which — through the pollen-tube — elements of the *hookeri*-plasmon will likewise come. By the somatical splitting of the hybrid plasmon manifold mixtures will be produced which will lethally act differently in the microspore. A pollen-population will arise which can be divided into three classes: active, inactive and empty grains.

Let us attempt to carry over our results, which are connected with the male gametophyte, into the sporophytic genome/plastom-system of Stubbe. When a genome *hookeri*, *franciscana* or *blandina* is incorporated into a mixture of Plastom I and Plastom III, a homogeneous pollen-population of viable grains is derived. Conversely, every plastom-mixture of plastom II *(purpurata)*, whether with I or III, produces a heterogeneous pollen-pattern with all the four genomes *(hookeri, franciscana, blandina* or *purpurata)*. This shows that the compatibility-conditions, which were proved by Stubbe between plastom and genome in the sporophyte are not entirely identical with the plasmongenom conditions in the ♂ gametophyte; here there appears e.g. no difference in the compartment of plastom I and III.

C — Pollen pattern of bastards between full homozygotes and semi-homozygotes.

Since the *rubrisepala*-forms of *Oe. coronifera* and *Oe. conferta* are isogametics, two hybrid-types appear in F_1: velutina and subvelutina.

(1) *Oe. coronifera rubs*-bastards.

All bastards have three pollen-classes, indepently from the course of crossings. The active grains are full, the inactives show all regression-forms, commencing from the grain hampered in its growth to the totally shrivelled, and empty grain (see Fig. 1). The limits between grain types are erased.

— Consequently *Oe. coronifera rubrisepala* behaves in the pollen-pattern as a heterozygotic species, though genetically and karyologically they are regarded as semi-homozygotes.

(2) *Oe. conferta-rubs.* bastards.

The results can be found in Table 2. Each group of reciprocal crossings represents its own case.

Both *purpurata*-hybrids show 3 pollen-classes, the lethality is, however, stronger here, than in the case of *purpurata*-bastards with full-mutants.

The reciprocal crossings with *blandina* provide the same situation, as found further above in the case of *hookeri* × *purpurata:* in one crossing direction 3 pollen-classes are shown by the bastard, in the other one a heterogeneous pollen pattern.

In all the hitherto mentioned hybrids no difference exists between the pollen-patterns of *velutina* and *subvelutina*. This is also the case with the bastard *conferta rubs.* × *hookeri;* in the *hookeri* × *conferta rubs.* reciprocal crossing, however, homogeneous pollen is shown by the *subvelutina* bastards and a heterogeneous pollen-pattern by *velutina* bastards. Here it is revealed, that the genomes *velans* and *subvelans* behave differently in the same plasmon with

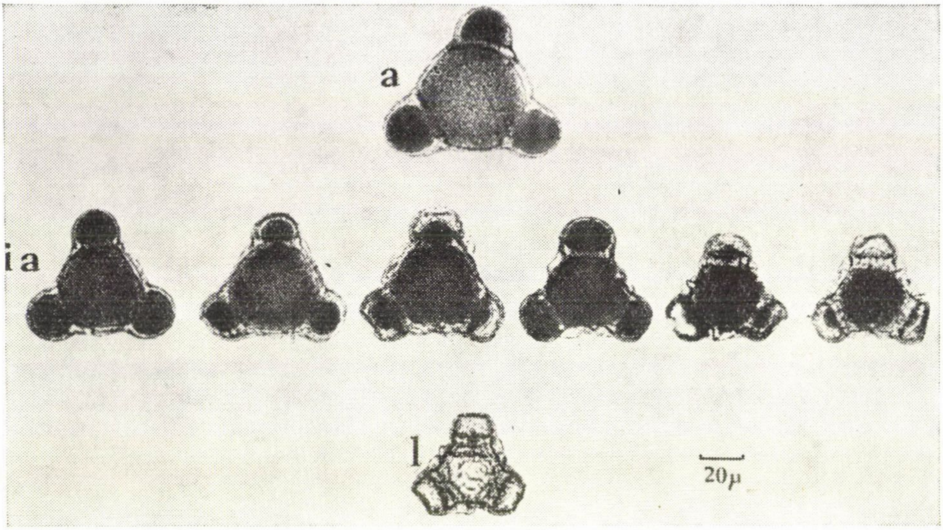

Fig. 1. Oe. coronifera rubrisepala × *Oe. purpurata* 3-class-pollens; The manifold inactive grains are arranged according to the regression-degree. a. active grain; ia. inactive grain; l. empty grain

Table 1

Pollen pattern of bastards between homozygotic Oenotherae

Bastards	Genome/plastom combination	Pollen pattern
Oe. hookeri × *Oe. franciscana*	^{h}hookeri/I, ^{h}franciscana/I	homogeneous
Oe. franciscana × *Oe. hookeri*	^{h}hookeri/I, ^{h}franciscana/I	homogeneous
Oe. hookeri × *Oe. blandina*	^{h}hookeri/I(+III), ^{h}blandina/I(+III)	homogeneous
Oe. blandina × *Oe. hookeri*	^{h}hookeri/I(+III), ^{h}blandina/III(+I)	homogeneous
Oe. blandina × *Oe. franciscana*	^{h}blandina/III(+I), ^{h}franciscana/III(+I)	homogeneous
Oe. franciscana × *Oe. blandina*	^{h}blandina/I(+III), ^{h}franciscana/I(+III)	homogeneous
Oe. hookeri × *Oe. purpurata*	^{h}hookeri/I(+II), ^{h}purpurata/I(+II)	heterogeneous
Oe. purpurata × *Oe. hookeri*	^{h}hookeri/II(+I), ^{h}purpurata/II(+I)	3 classes
Oe. blandina × *Oe. purpurata*	^{h}blandina/III(+II), ^{h}purpurata/III(+II)	heterogeneous
Oe. purpurata × *Oe. blandina*	^{h}blandina/II(+III), ^{h}purpurata/II(+III)	heterogeneous
Oe. franciscana × *Oe. purpurata*	^{h}franciscana/I(+II), ^{h}purpurata/I(+II)	heterogeneous
Oe. purpurata × *Oe. franciscana*	^{h}franciscana/II(+I), ^{h}purpurata/II(+I)	heterogeneous

Table 2

Pollen-pattern of bastards between full homozygote Oenotherae and translocation-mutants

Crossings	Bastards	Pollen pattern
Oe. conferta rubs. × *Oe. hookeri*	subvelutina	heterogeneous
	velutina	heterogeneous
Oe. hookeri × *Oe. conferta rubs.*	subvelutina	homogeneous
	velutina	heterogeneous
Oe. conferta rubs. × *Oe. blandina*	subvelutina	3 classes
	velutina	3 classes
Oe. blandina × *Oe. conferta rubs.*	subvelutina	heterogeneous
	velutina	heterogeneous
Oe. conferta rubs. × *Oe. purpurata*	subvelutina	3 classes
	velutina	3 classes
Oe. purpurata × *Oe. conferta rubs.*	subvelutina	3 classes
	velutina	3 classes

the same genome-partner *hookeri.* Thus it is hereby demonstrated that in the lethality-process the genome alone plays here together.

The bastards between homozygote *Oenotherae* show two kinds of pollen-patterns: either a homogeneous pollen-population with only full pollen-grains, or a heterogeneous population, where regressed and empty grains appear.

The same process determines regressed and empty grains, namely a lethality, which is plasmatically karyotically conditioned at the same time.

The share of the plasmon in the pollen-lethality is, however, prevalent, because between two homozygote parents which produce only full grains, only one bastard can come into being, which shows a heterogeneous pollen-pattern. The share of the genome in the pollen lethality can be established when two complexes arise from one of the parents (semi-homozygote).

The pollen-lethality in case of *Eu-Oenotherae* is therefore conceived as a disharmony between genome and plastom.

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INFLUENCE OF SOIL ORGANIC MATTER ON SIMULTANEOUS RELEASE OF SOIL NITROGEN AND PHOSPHOROUS

The solubility of soil nitrogen is influenced by several factors like soil moisture, soil temperature, pH, organic matter etc. Phosphate is next to nitrogen in increasing crop yield and according to BEAR (1942), within limits, phosphate fertilizers together with potassium and lime can substitute nitrogen fertilizers.

A recent work of BAINS (1961) indicates that the yield of crops in soils rich in available P, is favourably influenced by a nitrogenous fertilizer. SHANKAR—RAO (1966) showed that the utilization of soil N by plants is affected by the presence of soluble P, and P is essential for bacterial growth. HARGITAI (1967) studied the changes in nitrogen form in the soil and the mobility of soil humic acid by liming and found that nitrogen solubility is closely related to humus quality in a biochemical threshold range of soils at a critical pH range of 5.0 to 5.5 when PO_4 solubility approaches the maximum.

The purpose of this paper is to study the influence of organic matter on soil fertility status by the application of different organic matter fractions from soil humus with alternative additions of N, P and lime + N + P to organic matter free soils and thus to get an idea regarding the influence of organic matter fractions in soil in maintaining soil fertility.

Soils. Soil samples from the following localities mostly of West Bengal were collected for the present investigation.

Analytical methods. A. Kalimpong soil was chosen as the source of organic matter. The extraction and fractionation of humus compounds to humic, fulvic and haemetomelanic

Sample	Locality	Description
Kalimpong	District Seed Farm, Kalimpong, Darjeeling	0—15 cm depth alluvial soil
Burdwan sandy loam	Seed Multiplication Farm, Kalna, Burdwan	0—15 cm depth and 15—30 cm depth. Cultivated land of sandy loam type
Burdwan loamy soil	Kalna Block No. 1, Krishnadevpore, Dt. Burdwan	0—15 cm depth and 15—30 cm depth. cultivated plot of clay loam type
Padegaon black cotton soil	Sugarcane Research Institute, Padegaon, Dt. Poona	0—30 cm depth. Black cotton type
Jalpaiguri soil	Jalpaiguri farm	0—15 cm depth and 15—30 cm depth. Alluvial locality dark black in colour
Purulia soil	Purulia district farm	0—15 cm depth. Laterite zone

acid fractions were carried out by the usual method (BEAR 1968). The total organic carbon was determined by the oxidation method (JACKSON 1967d). The results are given in Table 1.

B) Total soil N was determined by the Kjeldahl method (JACKSON 1967c). Total soil P was determined by the HC104 digestion method (JACKSON 1967b). The results are given in Table 1.

C) For the determination of the extractable N in the soil, the procedure adapted by STANFORD (1968) was followed. The total available P was determined by Bray's No. 1 extractants (JACKSON 1967a). The results are given in Table 2.

Table 1

Total Soil C.N.P. and non-humic N, non-organic P in gms/100 gms of soil

Soil	C	N	P	Non-humic N	Non-organic P
Kalimpong (0—15)	1.68	0.2422	0.03	0.0833	0.01
Burdwan sandy loam (0—15)	0.9	0.0826	0.0375	0.0294	0.0087
Burdwan sandy loam (15—30)	0.876	0.0868	0.035	0.0308	0.0075
Burdwan loamy soil (0—15)	1.14	0.1008	0.0325	0.028	0.0062
Burdwan loamy soil (15—30)	1.001	0.084	0.0312	0.0258	0.007
Padegaon black soil (0—30)	0.16	0.0168	0.0237	0.014	0.005
Jalpaiguri soil (0—15)	1.4	0.1407	0.035	0.0826	0.0047
Jalpaiguri soil (15—30)	1.42	0.1426	0.0425	0.0854	0.0052
Purulia laterite soil (0—15)	0.95	0.0224	0.0125	0.014	0.005

D) The soil samples were freed from organic matter by extraction with 0.5N Na_2CO_3 and lastly by a treatment with 6% N_2O_2. In the organic matter free (O.M.F.) soils the total N (i.e. non-humic N) and total P (i.e. non-organic P) was determined by the usual procedure. The data are given in Table 1. The total extractable non-humic N and non-organic P was also determined by adapting the method mentioned in C. The data are given in Table 2.

E) The effect of organic matter and lime was studied next (1). To a known amount of O.M.F. soil known doses of organic matter fractions viz. humic acid (alkali soluble), fulvic acid (water soluble) and haemetomelanic acid (alcohol soluble) were added, this was followed by an addition of $(NH_4)_2SO_4$ as N source and CaH_2PO_4 as P source. The amounts of doses used depended on the initial C, N and P content of the soils. After proper moistening (to form a paste) the soils were kept for 3/4 days in stoppered conical flasks. Then the total soil P, soil N and total extractable N and P were determined in the soils. The results are shown in Table 3.

2. In another set of experiments only the N and P source were added without the addition of organic matter fractions to the O.M.P. soils. Then the total extractable non-humic N and available non-organic P were determined. The results are shown in Table 4.

3. In order to study the effect of lime on soil N and P solubility, lime was added to the soils mixed with 0.01M $CaCl_2$ at two different pH ranges 7.0 to 8.0 and 5.0 to 5.5, with the addition of a same amount of N, P and organic matter sources as in expt. E (1). The total extractable N and P in different organic matter fractions were determined as before. These results are reported in Tables 5 and 6, columns 1 and 2. Lime was also added with the N

Table 2

Cumulative total extractable and NaOH distillable soil N, non-humic N by 9 successive extns. with 0.01M $CaCl_2$ and available soil P, non-organic P by Bray's No. I extractants

Soil	Column (1)		Column (2)		Column (3)	Column (4)
	9 successive extns. with 0.01M $CaCl_2$. Amount of N in mgm/100 gms of soil		Amount of non-humic N in mgm/100 gms of soil by 9 successive extns. with 0.01M $CaCl_2$		Total available soil P in mgm/100 gms of soil	Total available soil non-organic P in mgm/100 gms of soil
	Total extd. N	Distillable NH_3-N	Total extd. N	Distillable NH_3-N		
Kalimpong (0—15)	4.333	1.319	1.105	0.322	2.475	0.725
B. sandy loam (0—15)	2.555	0.828	0.308	0.020	2.875	1.75
B. sandy loam (15—30)	2.639	0.892	0.044	0.032	2.25	1.75
B. loamy soil (0—15)	4.377	1.046	1.836	0.227	2.375	1.375
B. loamy soil (15—30)	4.093	1.116	1.097	0.237	2.375	1.365
Padegaon black soil (0—30)	3.533	1.085	0.718	0.158	1.25	0.375
Jalpaiguri soil (0—15)	4.128	1.319	1.048	0.231	4.00	1.025
Jalpaiguri soil (15—30)	4.284	1.426	0.996	0.278	3.00	0.875
Purulia laterite soil (0—15)	3.98	1.13	0.860	0.53	3.50	2.50

Table 3

Soil (O.M.F.) + organic matter fractions + N source as $(NH_4)_2SO_4$ + P source as CaH_2PO_4. Total soil N, extractable N, NaOH distillable N and total P available P in three different organic matter fractions

Soil	Amt. of O.M. fractions added in gms/20 gm soil	Amt. of N source added in gms/20 gm soil	Amt. of P source added in gms/20 gm soil	Total soil P in diff. O.M. fractions in gm/100 gms of soil		
				Humic	Fulvic	Haemeto-melanic
Kalimpong (0—15)	0.336	0.048	0.006	0.0137	0.0132	0.0125
B. sandy loam (0—15)	0.18	0.0165	0.007	0.016	0.01	0.01
B. sandy loam (15—30)	0.176	0.0173	0.005	0.015	0.012	0.01
B. loamy soil (0—15)	0.228	0.02	0.0065	0.01	0.0075	0.0075
B. loamy soil (15—30)	0.20	0.016	0.0062	0.013	0.0087	0.01
Padegaon black soil (0—15)	0.032	0.0033	0.0047	0.008	0.0087	0.01
Jalpaiguri soil (0—15)	0.28	0.028	0.007	0.01	0.0082	0.0062
Jalpaiguri soil (15—30)	0.284	0.028	0.0085	0.012	0.008	0.0075
Purulia laterite soil (0—15)	0.19	0.0044	0.0025	0.011	0.0075	0.0087

and P source without the addition of organic matter fractions to the O.M.F. soils and total extractable non-humic N and available non-organic P were determined. The results are reported in Tables 5 and 6, columns 3 and 4.

In the present experiment nine different soil types were taken. There is a large difference between the total soil N and soil P content before and after the removal of organic matter in these soils (Table 1). It is seen that humic N is always greater than non-humic N except in soils from Padegaon (black cotton type), Jalpaiguri (alluvial soil) and Purulia (laterite soil), these show the reverse order. Again, in the case of phosphorous, organic P is always greater than non-organic P. This reveals the fact that it is the organic matter that contains the major portion of the P and not the soil. However, in the laterite soils of Purulia the distribution of P is higher in the soil than in the organic matter. Similarly, there is a great difference in the soluble P and N before and after the removal of organic matter. The total extractable N (in initial soil) and the total extractable non-humic N (in O.M.F. soil) shows that (Table 2, column 1 and 2) humic N is always greater than non-humic N (obtained from the difference of column 1 and 2) for all types of soils. Organic P as obtained from the difference between the total available P and total available non-organic P (Table 2, column 3 and 4) is found to be less in amount in some typical soils viz. sandy loam, clay loam of Burdwan and laterite soil of Purulia.

The use of organic matter fractions in O.M.F. soils along with the P and N source (Table 3) shows that the humic acid fraction is more effective in releasing P when present in clay loam type soils of Burdwan and Padegaon and the laterite soil of Purulia, whilst the fulvic acid fraction is more effective when present in the two alluvial soils of Kalimpong and Jalpaiguri and the sandy loam soil of Burdwan. The effect of adding the haematomelanic acid fraction is not encouraging (Table 3, column 3). However, the humic acid fraction is found to be more effective for most of the soil types in releasing soil N and the fulvic acid

Total soil N in diff. O.M. fractions in gm/100 gms of soil			Extractable P in diff. O.M. fractions in mgm/100 gms of soil			Amount of extractable N in mgm/100 gms of soil by 9 successive extns. with 0.01 M $CaCl_2$ soln.					
						Humic		Fulvic		Haemetomelanic	
Humic	Fulvic	Haeme-tomelanic	Humic	Fulvic	Haeme-tomelanic	Total N Extd.	Dist. NH_3-N	Total N Extd.	Dist. NH_3-N	Total N Extd.	Dist. NH_3-N
0.091	0.096	0.10	2.50	3.025	2.25	12.04	9.313	12.32	9.79	10.00	9.00
0.036	0.036	0.039	2.125	2.50	2.00	11.13	9.976	9.92	8.47	8.34	6.24
0.037	0.04	0.042	2.00	2.525	2.00	11.40	10.04	10.21	8.74	8.97	7.00
0.049	0.058	0.065	2.875	2.50	2.375	8.25	6.82	9.37	7.90	7.35	5.55
0.050	0.065	0.068	2.75	2.50	2.375	5.55	4.265	9.36	8.03	7.785	6.05
0.023	0.036	0.062	3.00	2.45	2.25	13.08	12.84	6.09	4.725	8.02	7.00
0.085	0.065	0.099	2.75	3.025	2.50	13.30	12.88	13.288	11.902	11.32	9.55
0.086	0.066	0.105	2.75	3.05	2.525	13.22	10.284	13.74	11.90	11.57	9.57
0.018	0.017	0.015	3.25	2.625	2.875	4.22	0.284	2.93	0.98	1.52	0.9

Table 4

Soil (O.M.F.) + N source as $(NH_4)_2SO_4$ + P source as CaH_2PO_4. Cumulative total extractable non-humic N, NaOH distillable N and total available non-organic P

Soil	Total N in gm/100 gms of soil	Total P in gm/100 gms of soil	Available non-organic P in mgm/100 gms of soil	Amount of extractable non-humic N in mgm 100 gms of soil by 9 successive extrns. with 0.01M $CaCl_2$ soln.		Organic P in mgm/100 gms of soil, in different organic matter fractions			Humic N in mgm/100 ms of soil, in different organic matter fractions		
				Total N Extd.	Distillable NH_3-N	Humic	Fulvic	Haemeto-melanic	Humic	Fulvic	Haemeto-melanic
Kalimpong (0—15)	0.315	0.01	1.75	9.84	7.50	0.75	1.275	0.50	2.20	2.48	0.16
Burdwan sandy loam (0—15)	0.112	0.009	1.75	8.098	7.00	0.375	0.75	0.25	3.032	1.822	0.242
Burdwan sandy loam (15—30)	0.117	0.007	1.75	8.00	6.82	0.25	0.775	0.25	3.40	2.21	0.97
Burdwan loamy soil (0—15)	0.128	0.0068	2.25	7.02	4.05	0.625	0.25	0.125	1.23	2.35	0.33
Burdwan loamy soil (15—30)	0.109	0.007	2.00	5.00	4.00	0.75	0.50	0.375	0.55	4.36	2.785
Padegaon black soil (0—15)	0.0308	0.005	2.025	6.03	5.575	0.975	0.425	0.225	7.05	0.06	1.99
Jalpaiguri soil (0—15)	0.223	0.005	1.375	9.98	8.00	1.375	1.650	1.125	3.32	3.308	1.34
Jalpaiguri soil (15—30)	0.228	0.006	1.50	9.57	7.875	1.25	1.55	1.025	3.65	4.17	2.00
Purulia laterite soil (0—15)	0.036	0.005	1.25	1.49	1.40	2.00	1.375	1.625	2.73	1.44	0.03

fraction is especially effective for the clay loam type of soil of Burdwan, whereas the haemetomelanic acid fraction is the least effective in releasing both P and N (Table 3, column 4).

The organic P and N content of the soil (Table 4, columns 3 and 4) as has been evaluated from the difference between the total soluble soil P and/or N (Table 3, columns 3 and 4) and the total soluble soil non-organic P and/or N (Table 4, columns 1 and 2) show that when the humic acid fraction is present in soils of a clay loam or laterite type more of the P is in a soluble form and that when the fulvic acid fraction is present in an alluvial and sandy loam soil, the P becomes more soluble (Table 4, column 3). More organic N is present in the humic fraction when it is present in sandy loam, black cotton or lateritic soils, whilst the fulvic acid fraction contains more organic N when present in alluvial or clay loam soils. Here again the haemetomelanic acid fraction is found to be non-interfering (Table 4, column 4).

A study of the role of lime in releasing N and P from soils with or without organic matter show that at a pH of 7.0 to 8.0 a release of both N and P is always decreased by the addition of lime whether organic matter is present or not (Table 6), but as a pH of 5.0 to 5.5 the release of N and P is always greater both from soils with and those without organic matter (Table 5), which is in unison with the findings of Hargitai (1967).

Amongst the various organic matter fractions it was found that when the humic acid fraction was present more extractable N and P was obtainable from most of the soil types (Table 5, columns 1 and 2). The extractable non-humic N and P (Table 5, columns 3 and 4) is less due to the absence of the effective organic matter fractions. By taking the difference between the total extractable (i.e. by the addition of organic matter fractions) and extractable non-organic (i.e. without the addition of organic matter fractions), the organic P and organic N can be obtained. It was found that the humic acid fraction contained more organic N and P (Table 5, columns 5 and 6). These results show that liming at pH 5.0 to 5.5 helps in releasing most of the fixed N and P part in soils.

However, with the haemetomelanic acid fraction no observable improvement in the availability of N and P could be ascertained in either of the pH ranges.

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Table 5

With Lime at pH 5.0—5.5

Soil	Column 1			Column 2					
	Available soil P in mgm/100 gms of soil in different organic matter fractions			Extractable soil N in mgm/100 gms of soil in different organic matter fractions					
	Soil (O.M.F) + O.M. fractions + N. + P + lime			Soil (O.M.F) + O.M. fractions + N + P + lime					
	Humic	Fulvic	Haeme-tomela-nic	Humic		Fulvic		Haemetomelanic	
				Total Extd. N	Dist. NH_3-N	Total Extd. N	Dist. NH_3-N	Total Extd. N	Dist. NH_3-N
1	2	3	4	5	6	7	8	9	10
Kalimpong (0—15)	8.25	1.875	2.875	21.28	19.00	24.92	20.00	19.00	17.18
Burdwan sandy loam (0—15)	5.00	2.375	3.25	24.64	20.15	23.38	19.17	18.15	16.98
B. sandy loam (15—30)	4.75	2.525	3.475	25.48	20.00	23.80	19.00	18.00	16.00
B. loamy soil (0—15)	6.275	3.625	4.25	16.28	14.57	18.05	13.75	15.15	12.00
B. loamy soil (15—30)	6.125	3.875	4.05	17.49	13.00	17.82	13.12	14.98	11.95
Padegoan black soil (0—30)	7.125	3.475	2.875	13.16	11.28	11.75	9.55	13.00	10.00
Jalpaiguri soil (0—15)	8.475	2.375	3.025	29.39	26.30	26.18	22.02	20.00	17.00
Jalpaiguri soil (15—30)	8.375	2.50	3.25	29.96	26.00	25.90	22.00	20.15	16.95
Purulia laterite soil (0—15)	8.375	4.75	4.05	10.91	7.00	11.84	10.85	7.50	5.68

Table 6

With Lime at pH 7.0 to 8.0

1	2	3	4	5	6	7	8	9
Kalimpong (0—15)	2.75	3.375	1.875	17.50	15.00	14.98	12.00	16.07
Burdwan sandy loam (0—15)	4.00	3.05	3.25	20.72	18.01	12.20	9.98	13.57
Burdwan sandy loam (15—30)	4.25	2.75	2.80	20.00	18.00	12.00	9.00	13.00
Burdwan loamy soil (0—15)	2.50	2.55	2.525	11.57	8.50	11.00	9.05	9.57
Burdwan loamy soil (15—30)	3.475	1.50	1.475	11.00	8.00	10.55	9.00	9.01
Padegaon black soil (0—30)	5.025	4.25	3.975	10.98	8.15	10.00	8.05	8.00
Jalpaiguri soil (0—15)	3.70	4.70	3.375	18.75	16.02	15.50	13.00	17.95
Jalpaiguri soil (15—30)	3.375	4.00	3.625	18.00	16.01	15.00	12.98	17.00
Purulia laterite soil (0—15)	3.625	3.675	3.375	6.02	3.05	4.00	2.01	3.55

Column 3	Column 4		Column 5			Column 6		
Available soil non-organic P in mgm/100 gms of soil	Extractable soil non-humic N in mgm/100 gms of soil		Organic P in mgm/100 gms of soil in different O.M. fractions. (Difference of column 1 and column 3)			Humic N in mgm/100 gms of soil in different O.M. fractions. (Difference of column 2 and column 4)		
Soil (O. M. F.) + N + P + Lime	(Soil (O.M.F.) + N + P + Lime		Humic	Fulvic	Haemeto-melanic	Humic	Fulvic	Haemeto-melanic
	Total Extd. N	Dist. NH_3-N						
11	12	13	14	15	16	17	18	19
1.750	18.38	15.69	6.50	0.125	1.125	2.90	6.54	0.62
2.25	16.15	15.45	2.75	0.125	1.00	8.49	7.23	2.00
2.125	17.92	17.72	2.625	0.40	1.350	7.56	5.88	0.08
2.225	15.00	11.19	4.05	1.40	2.025	1.28	3.05	0.15
2.25	14.21	10.59	3.875	1.625	1.80	3.28	3.61	0.77
2.75	11.06	9.25	4.375	0.675	0.125	2.10	0.69	1.94
2.025	19.30	11.17	6.45	0.35	1.00	10.09	6.88	0.70
2.125	20.00	10.55	6.25	0.375	1.125	9.96	5.90	0.15
3.875	15.90	13.04	4.50	0.875	0.175	5.01	5.94	1.60

10	11	12	13	14	15	16	17	18	19
15.00	1.75	14.00	12.35	1.00	1.625	0.125	3.50	0.98	2.07
11.00	2.975	11.19	9.97	1.025	0.075	0.275	9.53	1.01	2.38
10.92	2.625	10.97	9.00	1.625	0.125	0.175	9.03	1.03	2.03
7.50	2.00	9.05	7.00	0.50	0.55	0.525	2.52	1.95	0.52
7.02	1.375	8.00	6.92	2.10	0.125	0.10	3.00	2.55	1.01
6.98	3.75	6.95	4.05	1.275	0.50	0.225	4.03	3.05	1.05
14.00	2.125	14.58	12.00	1.525	2.575	1.25	4.17	0.92	3.37
14.98	2.00	14.55	11.75	1.375	2.00	1.625	3.45	0.45	2.45
3.00	3.25	2.92	0.90	0.375	0.425	0.125	3.10	1.08	0.63

MORPHOLOGICAL VARIATIONS AND HERBICIDE SENSITIVITY OF CONVOLVULUS ARVENSIS L. IN THE WINE DISTRICT OF MÓR

In the course of a study on the weed biology of vineyards special attention was paid to an important weed species: the convolvulus. The question was dealt with according to the following aspects:

a) convolvulus, as the most important weed of vine,
b) the rich morphological variability of convolvulus,
c) convolvulus and the herbicides.

Convolvulus is dealt with by the botanical literature from vastly heterogeneous, though rather diversified aspects. In Hungary — and even when compared to the foreign literature — it is Soó's work (1968) that tries to make a synthesis of the most characteristic variants of this species.

At the beginning of the experiments the chemical control carried out in vineyards was referred to as the "Convolvulus problem". This much discussed problem remained an open question for years, and has been — and is even today — connected with a highly significant group of problems. Of them the most important ones are: the life form of weed plants (G_3), the properties and range of the herbicides applied, the composition of the weed flora on the areas studied, soil conditions, low doses applied initially on the basis of wrong suggestions made by the manufacturers (triazines) — (the latter circumstance may partly have been responsible for the phenomenon of a passive — and a supposed active — resistance in the weed plant in question, which have been discussed — and in many cases wrongly interpreted — in various lectures, publications and papers); the time and method of application, abiotic factors, etc.

The author first began to study the morphological variations of *Convolvulus arvensis* L. in 1959–1961 in "selected Convolvulus populations" developed on areas treated with triazine-type herbicides (Kiss 1964). Between 1962 and 1971 the morphological richness of convolvulus was studied on areas not treated with herbicides, in original weed populations of vineyards too (Kiss 1970).

Morphological variations of the species. In the course of the investigations climbing (*volubilis* Pohl 1810) and prostrate (var. *prostratus* Opiz 1841, Rouy 1908) forms were encountered without exception in vineyards grown on lime covered chernozem soil formed on loess, on skeleton, rocky and sandy soils, on mobile sand, dark lithomorphic forest soil, on the brown forest soils of Central- and South-East Europe, etc.

Characteristic leaf shapes: egg-shaped-ellyptic, with rounded shoulders (*oblongifolius* J. Murr. 1905), Vénhegy, Koronahegy, Babkert, Terézia, Márkushegy, Árpádhegy, Kantókúti, Szőkehegy, vine-hill at Pusztavám, Kopaszhegy, Krisztinahegy at Csókakő, Aranyhegy, Kotlókúti, the New Vineyards at Söréd, Tóhelydomb, Öreghegy at Csákberény, Gránási hill, Szessziós vineyards. Beside the characteristic leaf form mentioned there was a significant occurrence of the so called oval (*ovato-ellyptic* Mág. D. 1917) form in the former vineyards as well as in those at Vargakút, Tábor-, Öröm- and Remény hills, Kőhalom, Kertalja, Kálvária, Dávidhegy, Kantókút.

Other characteristic leaf forms are: heart-shaped ovate (f. *hastifolius* Lach 1829), lanceolate, sometimes with auricle (f. *sagittatus* Opiz 1841. Mág.-D. 1917. *sagittifolius* Fisch. 1810. Lasch 1829), linear lanceolate, sometimes with an auricle (f. *elatior* Gortani 1903), lobate with dentate auricles (f. *denticulatus* Opiz 1841).

Hairy leaf-type: (f. *pubescens*-, f. *villosus* Lej. 1813, Bolle 1865), on areas listed above.

Position of flowers on the stem: singly (*uniflorus* Opiz 1841), in twos (*biflorus* Opiz 1841), in groups of 3–5 (f. *multiflorus* Opiz 1841), on the areas listed.

Colour of flowers: white or pale pink (*leucanthos* Opiz 1841, *roseus* Opiz 1841), intensive pink (*purpurescens* Tacik 1963), with a purple crown or pale streaks in the throat of the corolla (*coronatus* Trautm. 1936, *radiatus* Soó 1966), corolla at the throat reddish with purple spots (*punctatus* Kiss 1968 Vénhegy!).

The form of the corolla: funneliform, round, entire pentagonous (f. *pentagonus* Soó 1966), corolla indented (f. *emarginatoides* Soó 1967), deep, widely triangular lobes (f. *triangularis* Soó 1966).

Convolvulus and herbicides used in vineyards. Susceptibility to herbicides in this weed species was studied in three ways:

1. by treating seedlings,
2. by treating plants at various stages of development,
3. in "time variation" experiments.

1. Convolvulus seeds collected in the year preceding the experiments, stored adequately and sown in spring into the vine rows at a depth of 2—2.5 cm germinated very well and began to grow. (Weed seeds getting deeper during soil cultivation maintain their germination ability for years.)

From the beginning of germination to the 4—8-leaf stage of the climbing stem (29—36 days after sowing) the convolvulus was found extremely reactive to all herbicides applied.

Even a tenfold dilution of farm-scale doses caused a complete destruction at this stage of the growing convolvulus. Convolvulus and other weed seedlings showed a similar reaction to the different mechanical interferences (raking, scuffling, hoeing, use of cultivator, etc.) too. By raking an area regularly every 16—18 days we were able to keep the so called cultivation control plots practically weedless over 70—80 days.

2. The developed weed plant of *Convolvulus arvensis* L. showed in many cases a significant morphological resistance to the farm-scale doses of various herbicides. The term "morphological resistance" is considered relevant as for example in the case of the root herbicides applied no "developed" resistance should be supposed to exist; it is the morphological, potential, locational and constitutional conditions of the weed plant in question that have to be taken primarily in consideration. Soil conditions (humus content, clay content, adsorption complex, impermeability, etc.) as well as precipitation and temperature can be regarded as the most important influencing factors. Thus the question has to be studied from the aspect of the dynamism of biotic and abiotic factors.

The remarkably high "virulence" of *Convolvulus arvensis* L. originates in all probability from the fact that its roots penetrating as deep as 2—3 m into the soil are able to satisfy the water and nutrient requirements of the plant even in periods of permanent drought. The root system possesses innumerable adventitious roots which — when broken up by soil cultivation — rapidly propagate.

The twining stem growing on the soil surface, even if temporarily destroyed by chemical or mechanical weed control, with the inactivation of the herbicide — or through regeneration — develops a new twining stem often in the same year. A similar phenomenon is encountered in the case of applying translocating or burning chemicals. However, some successful herbicide combinations are able to retain the development of convolvulus even for a whole vegetation period.

3. "Time variation" experiments. Some ten years ago we recognized how important it is to determine the time when the different doses of herbicides have an influence on the various weed species and their aspects. In order to study this question experiments were carried on for years with 3—4 replications, in which every three weeks from March on new treatments of identical dose were made. So our experiments were carried on throughout the whole year. We were able to follow with attention the susceptibility of the weed plant from

germination to the ripening of the seeds. The phenological development phases of the most important species could be compared in relation to several years. It was in this way that an acceptable foundation was laid down in some highly important questions.

These important experiments resulted in a solution found to suppress such a "difficult weed" as the convolvulus. According to the results of the experiments, in the case of the autumn application of Hungarian Hungazins, traditionally cultivated close spaced vineyards with medium heavy and heavy soils can be kept free of weeds for 3—4 years by 15—20 kg/ha applied (after a preparatory deep soil cultivation). No injuries can be observed on the vines, their development is unobjectionable, and above all spring work peaks can be considerably reduced, so the control operations remain within the limits of economicalness. Our results gave full evidence of the fact that the winter period did not considerably decrease the inactivation of the Hungazins. These investigations resulted in the remarkable finding that in high-cultivation Moser-cordon vineyards (and in other wide spaced vineyards too) some herbicides can be efficiently used against convolvulus if the treatments are performed at the stage of flowering.

On the basis of our experiments the herbicides used in vineyards can be grouped — with the more important ones emphasized — according to the following:

A) Root herbicides

1. Triazine derivatives. Hungazin PK (50 per cent Aktinit PK = chloro-ethyl-amino-isopropyl-amino triazine) (Atrazin, Gesaprim); Nikezin PK (50 per cent Aktinit PK); Aktikon (90 per cent Aktinit PK). The necessary doses of all three chemicals are determined by the quality of the soil and the composition of the weed flora. Efficiency is considerably influenced by the weather conditions. Adequate doses of preparations containing 50 per cent active agent range between 6 and 16 kg/ha, while of those containing 90 per cent active agent are 4—8 kg/ha. In the case of autumn application to impermeable soils 20 kg/ha was used efficiently of preparations containing 50 per cent active agent. Autumn application of triazines to sandsoils is not recommended.

Hungazin DT (Aktinit DT 50 per cent = chloro-bis-ethyl-amino triazine) (Simazin, Gesatop); Nikezin DT (50 per cent Aktinit DT). Owing to their relatively low solubility both preparations are recommended primarily for sandsoils, in doses of 5—6 kg/ha.

2. Carbamates. Telwar = Monuron = SMU (80 per cent chloro-phenyl dimethyl-carbamide). It was widely used in the United States and France. In our investigations it showed acceptable results thus in no case was it excluded from the experiments.

Aresin (50 per cent monolinuron = chloro-phenyl-metoximethyl-carbamide). It is a herbicide preparation of wide range. Vine shows adequate tolerance to it even when young. Therefore it can be used for the chemical weed control of vine nurseries. On sandsoils 10 kg/ha while on heavy soils 15 kg/ha proved to have a sufficient weed killing effect on convolvulus.

Afalon (50 per cent linuron = di-chloro-phenylmetoxi-methyl-carbamide). Using the same doses it was proved to be more efficient than Aresin in all experiments. Can similarly be used in vine nurseries. It was found to exercise a satisfactory effect on convolvulus.

When using either Aresin or Afalon we found that their range of action considerably exceeded that of the triazin derivatives. They proved efficient against rhizomatic weeds and convolvulus both in small plot- and farm-scale treatments. Their only disadvantage is their having a shorter than necessary weed killing effect which does not last to the end of the vegetative period.

3. Benzonitryl derivatives. Prefix granulate (7.5 per cent chloro-thiamide = dichlorothiobenzamide). Using a dose of 150 kg/ha showed a good weed killing effect lasting for 65—70

days both in genuine (not yet treated with herbicides) and "selected" convolvulus populations. Its farm-scale application is suggested to be carried out with a VICON-type fertilizer distributor.

B) Contact herbicide preparations

Regarding their action mechanism they can be called scorching preparations.

Gramoxone (25 per cent paraquat-dichloride = dimethyl-dipiridyl). The preparation is used as a solution of 0.4—0.5 per cent. Its dose is 4—5 liter per hectare. In the course of the treatment the weeds wither in a few hours; perennial weeds develop a new growth within 14—16 days. According to our observations the weed killing effect is of a somewhat longer duration than in the case of mechanical weed control. In Moser-cordon- and other wide spaced vineyards the means of application is the "Gramospray 4239" row spraying frame drawn by the special UE-28-, MTZ-, Super Zetor-50-, etc. tractors. During a vegetative period 3—6 row treatments are performed, so the amount and cost of application are reduced to one-third. The latter applies to all row and strip treatments performed in vineyards.

C) Combined herbicide preparations

Numerous foreign herbicide combinations have been tested for years in our vineyards. Of them only a few are mentioned here, without any detail given as to their composition: Saminol A-1089, Domatol-A-1089, Domatol SW-A-2086, Campaprim A-1544, Semparol-A-1167, Vinipan, etc. All the herbicide combinations mentioned also contain components of hormonal effect; among them there are excellent preparations against convolvulus too.

Afalon Special H 2839 (= linuron + monolinuron) (50 : 50 per cent). It is considered to be an excellent herbicide combination of wide range of action for vineyards. It keeps the vineyard free of weeds throughout the vegetative period. It has been efficiently used on areas covered by convolvulus too, at doses of 14—15 kg/ha on medium heavy soils.

Buvinol (25 per cent PK + 25 per cent Klorinol). It is a well proved Hungarian herbicide combination. Its range of action is equal to any of the herbicides mentioned. Its moderate price makes its large-scale application possible. Its nation-wide acknowledgement as a vine herbicide is increasing. The suggested doses range between 10 and 18 kg/ha, depending on the permeability of the soil. Convolvulus is efficiently suppressed by the usual spring (March, April) application, but with an adequate technology can be successfully used at the stage of flowering as well.

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EFFECT OF PLOT SIZE ON THE RELIABILITY OF THE EXPERIMENT

In plant breeding the field experiments are aimed at testing the productivity of the different strains. The reliability of the experiment is influenced by many factors. As in many cases only small quantities of seed are available for the breeder which are not sufficient for starting large plot experiments, he has to know the minimum plot size and number of replications by which reliable and possibly full value results can be obtained.

According to Sváb (1967) the plot size depends on the homogeneity of the soil. In Hungary the breeders generally lay out comparative trials with 4—6 replications on plots of 12—20 m^2 each. For cereals I'Só—Berzsenyi-Janosits (1961) suggest the lower limit of the plot size to be 2—4 m^2. To study the agronomic characteristic of wheat "A" strains Rajki (1964) and Balla (1970) even used plots of 0.5 m^2, in four replications.

In the Soviet Union comparative trials are performed on 100 m^2 plots in 4—6 series (Molostov 1966). Dospehov (1968) considers 10—40 m^2 plots suitable for experiments performed with 4—6 replications while mentioning that many breeders use as small plots as of 0.5—2.0 m^2 if they have not enough seed.

Smith (1938) suggested to use plots of 0.45 m^2, while Elliot *et al.* (1952) 2.5 m long plots with 4 rows spaced at 30 cm for the evaluation of the basic breeding material. According to Frey (1965) hill plots can be efficiently used for studies on the early generations of cereals, however, the final evaluation of strains must be carried out in row plots. He does not give the dimensions of the row plots. In the case of a lack of space, low quantity of seed (early generation, hybrid wheat) or cost reduction Jensen—Robson (1969) suggest the use of miniature linear hill plots at the rate of 1 g of seed per row.

On a given area the reliability of the experiment increases and the variability of yield decreases when the plot size is increased (Molostov 1966, Salmon—Hanson 1970). The plot size depends on the level of mechanization too. Large plot experiments are more like large-scale wheat growing (Dospehov 1968). Several authors (Roemer 1939, Mudra 1958, Kudryavtzeva 1959, Dospehov 1968) arrived at the conclusion that the higher number of replications had more to do with the reliability of the experiment than the larger plot size.

Since the researchers' opinions about the plot size are different we thought it reasonable to study this problem under Hungarian conditions in detail, mainly in consideration of the efficiency of breeding work.

Accordingly we studied the correlations between

a) plot size and reliability of experiment;

b) yield data of various size plots.

Our experiments were carried out at the Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár in 1970 and 1971, with 17 improved strains and a control variety (Bezostaya 1). The soil was a medium heavy loam in good condition. Peas with sunflower were grown as a forecrop. Fertilizers of N_{120}, P_{100}, K_{80} active agent content per hectare were applied a year. Seed grains were sown each year into a carefully prepared soil, at an optimum time (5—8 October).

The experiments were carried out with three plot sizes:

1. large plot	15.6 m^2
2. small plot	5.0 m^2
3. micro-plot	0.5 m^2

In plots 1. and 2. seeding rate of 550—600 germinable grains/m^2 was used, which is also used in practice. In the micro-plots 80 grains were sown with a spacing of 10×5 cm, which is somewhat less than the standard amount of seeding rate. In the large plot experiments 13, in the small plot ones 6 and in the micro-plot experiments 4 rows were sown per plot. The first two treatments were sown mechanically, the third by hand, in a random block design with six replications. The features of the experiments correspond to those used at our Institute with strains A, B and C.

The weather conditions were favourable for wheat production. To determine the reliability of the experiment the following parameters were employed. Significant difference (S.D. %) for which we accepted the internationally used P: 5% as the probability level of statistical evaluation. Variation coefficient (CV %) generally acceptable between 6 and 14 per cent (Sváb 1967). If the value of the variation coefficient is below 10 per cent the variation is low, between 10 and 20 per cent it is medium, while above that high (Dospehov 1968). Error of mean value ($S\bar{x}$ %). In field experiments if the value of $S\bar{x}$ is 1—2 per cent, the result is excellent, 3—5 per cent is good, 6—7 per cent satisfactory (Dospehov 1968). The closeness of correlation, its significance is expressed with the correlation coefficient (r), while the regression correlation with a linear regression equation ($Y = a + bx$).

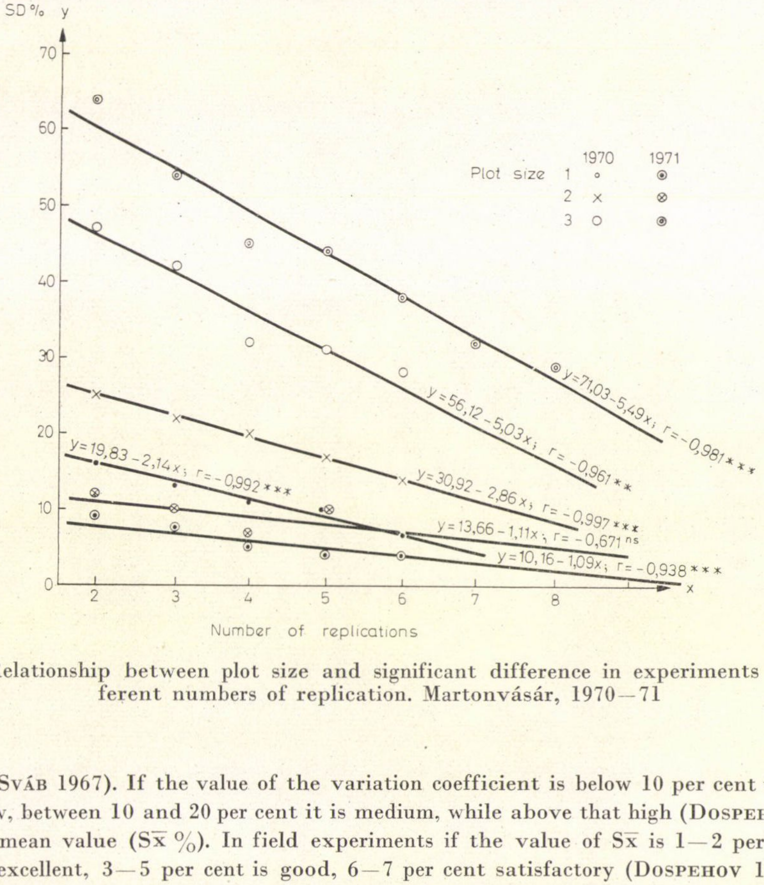

Fig. 1. Relationship between plot size and significant difference in experiments with different numbers of replication. Martonvásár, 1970—71

The experiments were successful in both years, however, in 1971 the parameters gave evidence of a higher reliability with plot sizes 1. (15.6 m^2) and 2. (5 m^2) (Table 1). With plot size 3. (0.5 m^2) relatively modest results were obtained both in 1970 and 1971. The above told are also confirmed by the data of the diagram.

Of the experiment types employed the large plot experiment is considered the most reliable, therefore the reliability of the small- and micro-plot experiments can be judged by a comparison to the large plot experiment. In the large plot experiments the values of SD %, $S\bar{x}$ % and CV %, under the soil and weather conditions of Martonvásár, were similar

Table 1

Changes in the values of SD 5%, S$\bar{x}$ % and CV % as a function of the number of replications and plot size (in the per cents of values)

Plot size	Year	Indices	Number of replications				
			2	3	4	5	6
1	1970	SD 5%	15.8	12.9	11.2	9.7	6.7
	1971	SD 5%	8.5	6.8	5.2	4.1	4.4
	1970	S$\bar{x}$ %	4.6	3.8	3.3	3.0	2.3
	1971	S$\bar{x}$ %	3.0	2.5	1.9	1.5	1.9
	1970	CV %	6.5	6.8	6.6	6.9	5.5
	1971	CV %	4.2	4.4	3.8	3.6	4.7
2	1970	SD 5%	25.3	21.9	20.0	16.5	13.7
	1971	SD 5%	12.3	10.1	7.1	9.6	—
	1970	S$\bar{x}$ %	9.1	9.8	6.0	1.5	1.2
	1971	S$\bar{x}$ %	3.8	3.4	2.7	3.4	—
	1970	CV %	13.1	10.9	11.7	11.2	10.1
	1971	CV %	5.7	5.8	6.0	7.5	—
3	1970	SD 5%	47.3	42.0	32.1	30.9	27.7
	1971	SD 5%	63.5	53.6	45.0	44.0	38.0
	1970	S$\bar{x}$ %	12.1	11.0	9.6	8.7	7.6
	1971	S$\bar{x}$ %	18.1	15.3	13.4	11.8	11.8
	1970	CV %	17.3	19.7	19.6	19.7	18.8
	1971	CV %	25.9	26.2	26.1	27.3	27.3

to those obtained in the previous year, while the values of small- and micro-plot experiments were higher than those, so they may give more reliable results in other years.

When examining the reliability of the small-plot experiment we find that the SD value attained in it with five replications could be attained in the large plot experiment already with two replications. Values showing the reliability of the experiment are still higher — that is: worse — in the micro-plot experiments. It can be established that — in the range of plot sizes studied — with an increase in the plot size the variability of yield decreases and the reliability of the experiment increases. The values of SD and S$\bar{x}$ percentage show similar trends, while the CV % decreases up to the fourth replication, then slightly increases, which can be attributed to the heterogeneity of the soil. It must be noted that the higher SD value in the small- and micro-plot experiments is to some extent counterbalanced by the fact that in these experiments there was a greater difference between the varieties.

Thus, within the range of the plot size studied, the reliability of the experiment can be improved by an increase in the number of replications. According to our data, taking a six-replication experiment as a basis, when the unit plot size (0.5 m^2) is increased tenfold and thirty one-fold, the reliability of the experiment will be 2.08-times and 4.13-times as high as before, respectively. In experiments with two and four replications, respectively, the increase of reliability shows similar trends (Table 2).

Table 2

Trends in the reliability of the experiment (SD %) as a function of plot size and number of replications

Plot size m²	Relative number							
	between plot sizes	in the case of			between			
		2	4	6	2	2–4	4–6	2–6
		replications, according to the plot size			replications			
0.5	1.0	100	100	100	100	147	115	171
5.0	10.0	186	160	208	100	126	145	184
15.6	31.2	299	286	413	100	141	167	235

Table 3

Correlations between results obtained with different plot sizes

Year	Plot size	r	r²	a	b
1970	1—2	0.828***	0.685	0.55	0.19
	1—3	0.407^{0}	0.165	5.97	1.56
	2—3	0.488*	0.238	1.62	8.08
1971	1—2	0.560**	0.313	1.63	0.12
	1—3	0.178^{NS}	0.031	0.12	0.01
	2—3	0.698***	0.487	—0.04	0.07

*** significant at 0.1% level
** significant at 1.0% level
* significant at 5.0% level
0 significant at 10.0% level
NS non significant

If we increase the number of replications from two to four (twofold), the reliability of the experiment will increase 1.2—1.5-fold, and when we increase it from to six (threefold), the reliability of the experiment will be 1.7—2.3 times as high as before, depending on the plot size.

On the basis of the data obtained in the years examined reliable correlations were found between the yield data of plot size 1. and 2. as well as of plot size 2. and 3. Between the yield data of plot size 1. and 3. there was no significant correlation (Table 3).

This shows that the 15.6 m² plots satisfy all the demands of the breeders. Apart from their giving reliable results, they are completely mechanizable from sowing to harvesting. However, such six-replication experiments require some 4.5—5 kg seed grain.

Results obtained in 5 m² plots with six replications were also fairly reliable. The higher SD value is more or less counterbalanced by the greater difference between the varieties. According to the results of several years trials the value of SD can be reduced to 8—10% by improved cultural practices. Therefore, if the breeder has 1.0—1.3 kg seed grain at his disposal, this experiment is recommended.

The value of the 0.5 m^2 micro-plot experiments is disputable. The high SD value is not counterbalanced by any greater difference between the varieties, and the evaluation of the data is made difficult by a border effect too, therefore this type of experiment is recommended only in such cases when no more seed grains are available and optimum conditions can be ensured for wheat growing.

In the micro-plot experiments much care must be taken when selecting on the basis of the yield, as indicated by the values of significant difference, $S\bar{x}$ % and variation coefficient, on one hand, and by the lack of correlation between the yields of large- and micro-plot experiments, on the other. 0.5 m^2 plots are most reasonably used for the yield comparisons of early generations, or for hybrid wheat experiments where in most cases very small amounts of seed grains are available for the breeder. Careful work is of great importance, as the loss of but a few plants influences the yield of the plot to a great extent, increases the scatter, the value of the variation coefficient, the significant difference — in one word, impairs the correctness of evaluation.

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PHYTOCHEMICAL STUDY ON FERULA MARMARICA ROOTS

Poisonous plants when eaten by animals cause harmful effects which in some cases may lead to death. Such plants are mostly distributed in fields, waste grounds and deserts.

These plants therefore may cause economical losses in the animal wealth leading to decrease in both production (meat, milk, wool and skin) and reproduction.

Among these poisonous wild plants is *Ferula marmarica* that belongs to the family Umbelliferae (TÄCKHOLM *et al.* 1954, MONTASER — HASSIB 1956).

Much work has been published on other Ferula species grown elsewhere (ALLPORT 1943, WILLIS 1951, WARM 1956) but nothing was recorded on *Ferula marmarica.*

The prevalence of this plant in the Egyptian western desert and the possibility of producing toxicity in farm animals especially sheep, suggested the studying of its chemical constituents.

Ferula marmarica plants were collected during January 1968, west of Alexandria, at 176 km along the desert coastal road from the area extending between the desert highway and the railway.

The plant was divided into its different parts, each part was air dried, separately ground and preserved for further investigation.

The determination of different properties was carried out on the roots according to the Egyptian Pharmacopoeia 1953, (AOAC) Official methods of Analysis 1955, and PAECH—TRACEY (1956).

Overall characters of *Ferula* roots. The roots were found to have a total moisture content of 77.77%, relative moisture of 2.66%, ash content of 12.68%, acid insoluble ash of 2.47%, total nitrogen of 0.94%, crude protein of 5.88%, crude fibres of 14.24%, and nitrogen-free compounds of 56.87%.

On extraction with successive solvents, the percentage of residue after distilling off the solvent was 7.67% with petroleum ether, 0.44% with diethyl ether, 0.64% with chloroform and 8.56% with ethyl alcohol. No crystalline compounds were obtained in the different extracts.

The roots were found to contain sterols, reducing substances, carbohydrates and/or glucosides, resins and saponins.

Chromatographic screening of the root extract. A paper chromatographic technique was employed for the screening of the crude alcoholic extract of the root using different reagents in spraying the developed chromatograms for qualitative aspects of the colour to help in identification.

After the development of chromatograms in different solvents such as n. butanol : glacial acetic acid : water (4 : 1 : 5) according to SMITH 1962, one paper was sprayed with ninhydrin which was prepared according to the technique described by LEDERER—LEDERER (1957) and SMITH (1962) for identification of amino acids.

Another paper was sprayed with Dragendorff's reagent modified by adding tartaric acid in place of acetic acid for detection of alkaloids.

A third paper was sprayed with aniline for detection of sugars.

The fourth paper was exposed to iodine vapour for identification of unsaturated compounds.

Another set of Whatman No. 1 papers were developed in n. butanol : pyridine : ammonia 3% (7 : 3 : 10). The first paper was impregnated with 10% antimony trichloride in chloroform then dried at 105°C for 3 minutes for detection of glucosides, the second one was sprayed with a saturated solution of 2.4-dinitrophenyl hydrazine for detection of ketonic compounds according to VEIBEL STIG (1954) and the third paper was exposed to ammonia vapour and examined in day and ultraviolet lights according to BLOCK *et al.* (1955) for detec-

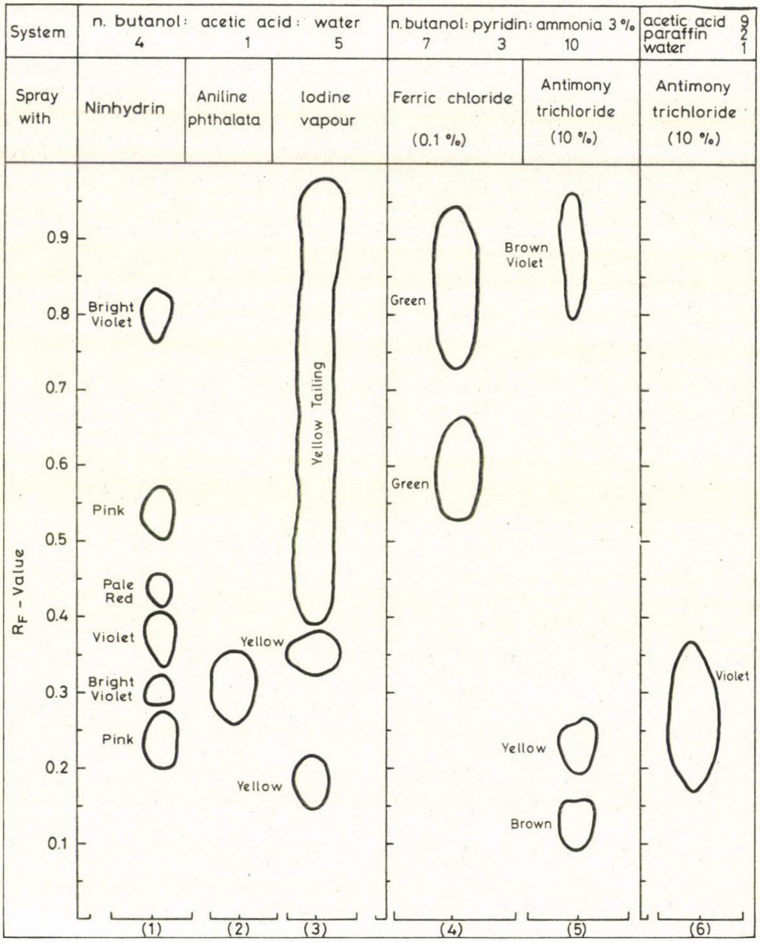

Fig. 1. Paper chromatographic of the alcoholic extract from *Ferula marmarica* root (1. amino acids, 2. free sugars, 3. unsaturated compounds, 4. phenolic compounds, 5. glycosides, 6. sterols)

tion of flavonoids. A fourth paper was used for the detection of phenolic compounds by spraying with ferric chloride.

Another set of Whatman No. 1 papers were impregnated with 10% liquid paraffin in benzene and developed in glacial acetic acid : liquid paraffin : water (9 : 2 : 1), then dried and impregnated with 10% antimony trichloride for detection of steroidal compounds.

From the illustration of the chromatographic screening (Fig. 1) it can be observed that the alcoholic extract of the root may contain 6 spots of amino acids with R_f-value 0.24, 0.30, 0.37, 0.43, 054 and 0.80 respectively; one spot of free sugar of R_f-value 0.31; three spots of glycosides with R_f-values 0.12, 0.23, 0.87 respectively; a spot of sterol R_f-value 0.26; two spots of phenolic compounds with R_f-value 0.59 and 0.83 respectively; and two spots of unsaturated compounds with tailing having R_f-value 0.18 and 0.35 respectively.

No alkaloids, ketones or flavonoids could be detected.

On screening the shoot, it was observed that it contained one glucoside.

Crystalline compounds from the root of *Ferula marmarica.* During the different trials of extractions with different solvents, a crystalline compound was isolated in benzene. Extraction: 200 g of the air dried root powder were extracted with benzene in the continuous extraction apparatus for about 18 hours until complete exhaustion. The residue was dissolved in the least amount of dry benzene and left at room temperature where a precipitate appeared. The benzene layer was decanted and the precipitate was washed several times with cold benzene and dried. 0.787 g of yellowish crystalline substance of granular appearance was obtained.

Characters of the crystalline fraction of benzene extract from *Ferula marmarica.* It was found that the compound had a crystalline form and a bitter taste, giving a positive reaction with Lieberman test and a negative with Molisch's test. It did not reduce Fehling solution, but reduced potassium permanganate in both acid and alkaline media. It gave a positive reaction to ketones with 2.4-dinitrophenylhydrazine and sodium nitroprusside tests indicating that it may contain a carbonyl group, but negative reaction with both ferric chloride solution and Wagner's reagent. Moreover, it did not reduce ammoniacal silver nitrate. Charring occurred when treated with sulphuric acid but no change occurred either with sodium hydroxide or with 1 N. hydrochloric acid. The test with magnesium turning for flavonoid was also negative.

The compound was soluble in hot ether, petroleum ether, chloroform, ethyl alcohol, benzene, methyl alcohol, acetone and pyridine but not in the cold solvents. It was insoluble in both hot and cold.

The melting point of the crude extract was 79—82°C. On re-crystallization in diethyl ether, the crystals had a melting point of 83–85°C.

The sodium fusion test revealed that the compound did not contain nitrogen, halogen, or sulphur and the test for phosphorus was also negative.

A microanalysis of the compound revealed that it contained C: 77.10 and H: 9.11.

The molecular weight of the compound was 328.9 with a calculated molecular formula of $C_{21}H_{30}O_3$. It was named "Marmarin".

The ultraviolet spectrum of the substance determined in a sp. 700 spectrophotometer (Unicum, Instruments Ltd.), (Fig. 2) showed maximum absorption at 284.9 mμ and this confirms that it is an aromatic compound.

The infrared spectra were determined in an I. F. Beckman model I.R.4, Range 1—15 μ. The graph showed a characteristic band of the ketonic group at 5.75 μ and substitution bands at 7.5—8, 8.4—9, and 9.4—9.8 μ, whereas at 10—11 μ a characteristic band of the aromatic group and its substitution at 13.3—14.2 μ as shown in Fig. 3.

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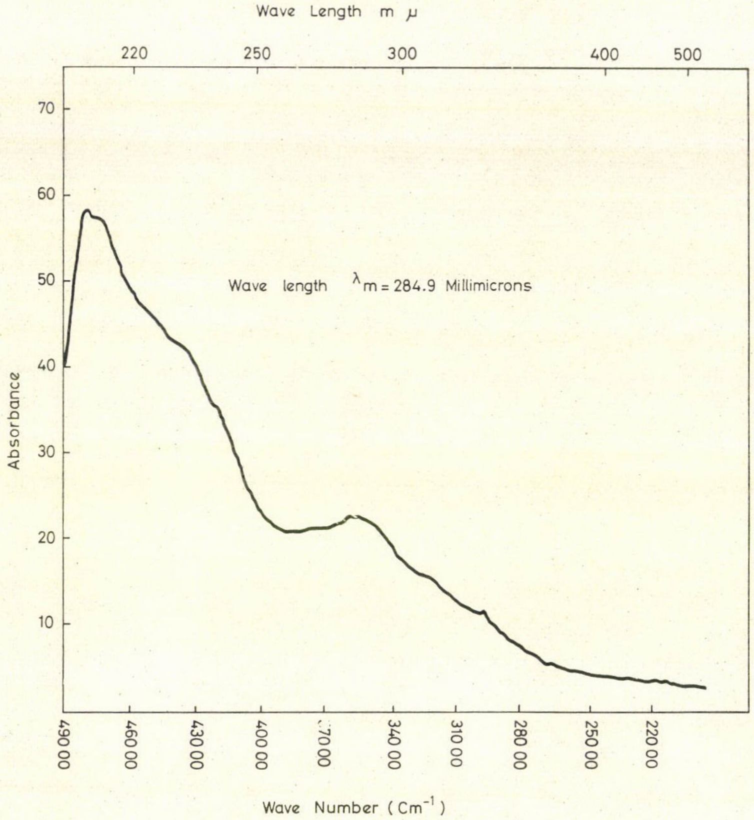

Fig. 2. Ultraviolet absorption curve of crystalline compound from *Ferula marmarica* root

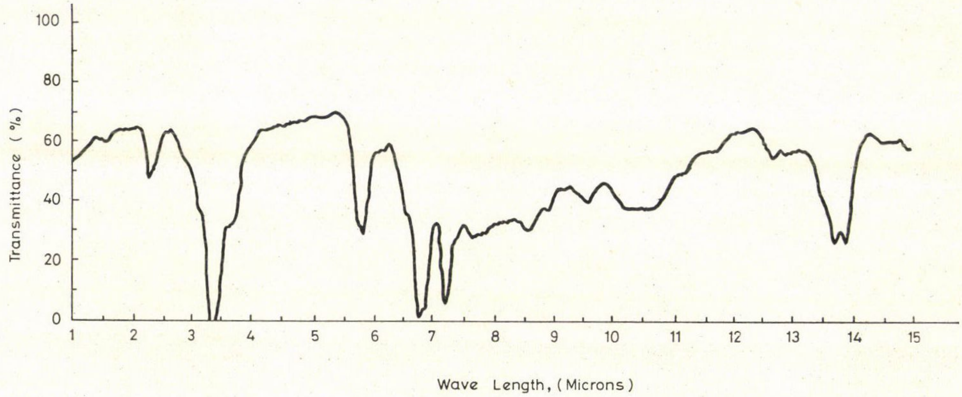

Fig. 3. Infrared spectrum of the separated crystalline compound from *Ferula marmarica* root

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TYPES OF INDUCING HAPLOID EMBRYOIDS AND PLANTS FROM IN VITRO ANTHER CULTURES

Since the results obtained by GUHA—MAHESHWARI (1964) many authors have succeeded in raising haploid plants from anther cultures (HESZKY 1971). Haploid callus- and embryoid formation could be induced from the anthers of gymnosperm and angiosperm (dicotyledon, monocotyledon) plant species and of their hybrids too. Both literary and our own results show that there are different ways of producing haploid plants from anther cultures, which the researchers have described partly separately. On the basis of results obtained in the last several years the paper tries to summarize and outline the possible types of inducing haploid embryogenesis, emphasizing the easiest and most reliable methods available for plant breeding and genetic research.

Development processes other than sporo- and gametogenesis, with some species in situ, with others in vitro, are summarized in Table 1 together with the plant species examined and the authors describing them. The table shows that there are different ways of producing haploid plants from anther cultures, as confirmed by the investigation results of the last years.

Figure 1 presents the possible ways of haploid embryo- and plant induction as well as the development processes differing from the sporo- and gametogenesis. The numbers shown in the figure refer — besides the caption — to the numbers in the first column of Table 1.

In plant species belonging to the family *Liliaceae* the macrogametophyton developing from the microspore mother cell (Fig. 1/1—3) can generally be observed *in situ*. This process proves that the development of microspore mother cells does not always result in microspores. It is partly due to this fact that in the anthers of *Nicotiana tabacum* — partly in situ, partly in the first week following isolation — there can be found embryoid formations consisting of 6—16 cells. According to HESZKY—PAÁL (1972) this process is the result of the haploid cells produced during the sporogenesis becoming embryonary under the influence of the biologically active substances of the anther tissues (Fig. 1/21—22—23).

Another — similarly callus-free — form of haploid embryoid induction is the development of the embryoid from the multicellular pollen grain (Fig. 1/12—13—14—23). Development of multinuclear pollen grains in the isolated anther indicates disturbances in the gametogenesis (Fig. 1/4); cell-walls formed around the nuclei are responsible for the appearance

Table 1

Possible ways of microspore development in anthers of various plant species

Reference number of Fig. 1	Pathways of microspore development	Plant species	Author
1—3	pollen-embryo-sac from microspore mother cell (in situ)	*Hyacinthus orientalis* *Leptomeria billardieri*	Stow 1934, Geitler 1941 in Maheshwari—Rangaswamy 1965, Ram 1959
4	multinuclear pollen grain from microspore in gametogenesis (in vitro)	*Trillium erectum*	Sparrow—Pond—Kojan 1955
2—6	hypertrophic pollen grain from pollen grain (in vitro)	*Nicotiana tabacum* *Lolium* × *Festuca* hybrid	Nakata—Tanaka 1968, Heszky—Paál 1972, Nitzsche 1970
2—8—9	haploid callus induction from germinating pollen grain (in vitro)	*Gingko biloba*	Tulecke 1953, 1957
18—19—20—23	haploid callus and plant from microspore in sporogenesis (in vitro)	*Nicotiana tabacum*	Nakata—Tanaka 1968, Heszky—Paál 1972
15—16—17—23	haploid callus and plant from microspore in gametogenesis (in vitro)	*Datura inoxia* *Orysa sativa* *Lolium* × *Festuca* hybrid	Guha—Maheshwari 1964, Niizeki—Oono 1968, Nishi—Mitsnoka 1969 Nitzsche 1970
21—22—23	embryogenesis induction from haploid cell, without callus formation (in vitro)	*Nicotiana tabacum*	Nakata—Tanaka 1968, Nitsch—Nitsch 1969, Sunderland—Wicks 1969, Maliga—Szilágyi 1971, Heszky—Paál 1972
12—13—14—23	haploid embryo and plant from multicellular pollen grain (in vitro)	*Datura stramonium*	Guha—Maheshwari 1966, 1967

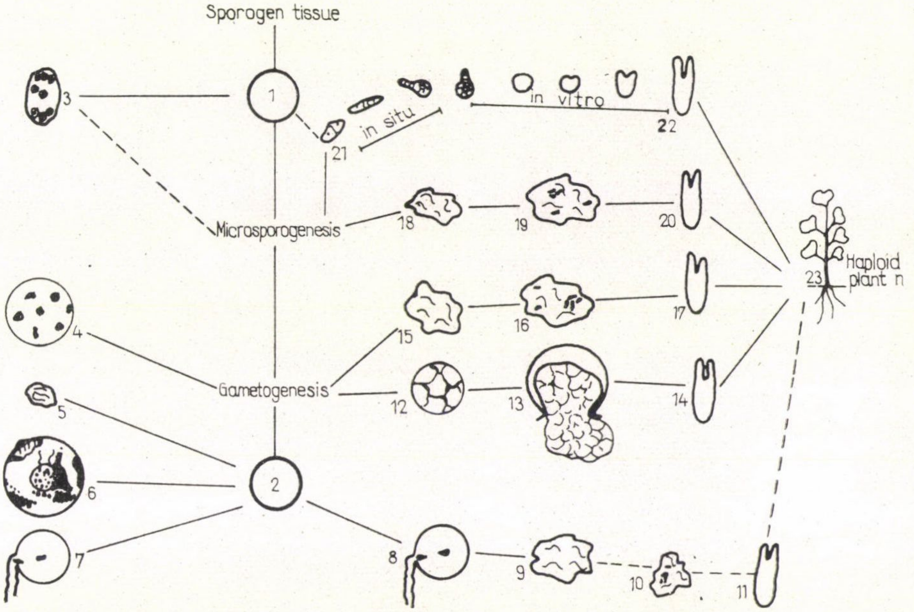

Fig. 1. Possible ways of in vitro microspore development in anthers of various plant species [1. pollen mother cell; 2. pollen grain (mature); 3. pollen-embryo-sac; 4. multinuclear pollen grain; 5. shrivelled pollen grain; 6. hypertrophied pollen grain; 7. germinated pollen grain; 2-8-9. callus induction from germinating pollen grain; 12—13. embryogenesis from multicellular pollen grain; 15—16—17., 18—19—20. callus and embryogenesis or organogenesis induction; 21—22. embryogenesis from haploid cell; 11-14-17-20. embryoid or organ induction.]

of multicellular pollen grains (Fig. 1/12) in the anther cultures of *Datura* species. Both in *Nicotiana* and *Datura* species embryoid development starts without callus formation which can be brought into connection with the frequency of the apomixis (haploid parthenogenesis, androgenesis) characteristic of these genera (Fig. 2).

In anthers placed on the culture medium most of the pollen grains germinate, wither or become hypertrophic (Fig. 1/2—5, 2—6, 2—7). These pathways of development generally do not result in morphogenesis. In exceptional cases in angiospermous species callus formation can be induced from germinating pollen grains (Fig. 1/2—8—9) which process can probably be explained by the analogy of androgenesis. The broken line (Fig. 1/2—10—11—23) shows an organization which, though induced in vitro, has not been demonstrated experimentally.

According to the results of investigations made so far the most feasible way of producing haploid plants, both in dicotyledonous and monocotyledonous plant species, is to induce haploid callus formation from microspores partly in the sporogenesis (Fig. 1/18—19—20—23), partly in the gametogenesis (Fig. 1/15—16—17—23) as a first step, then raise haploid plants from the callus by organogenic induction. It is supposed that with this method, by placing anthers isolated at the proper stage of development on culture media ideal for the plant species concerned, haploid forms of an increasing number of plant species will be produced (Fig. 3, Fig. 4). The most recent results support this expectation as well since haploid callus has been successfully induced and haploid plant raised in anther cultures of *Triticum*

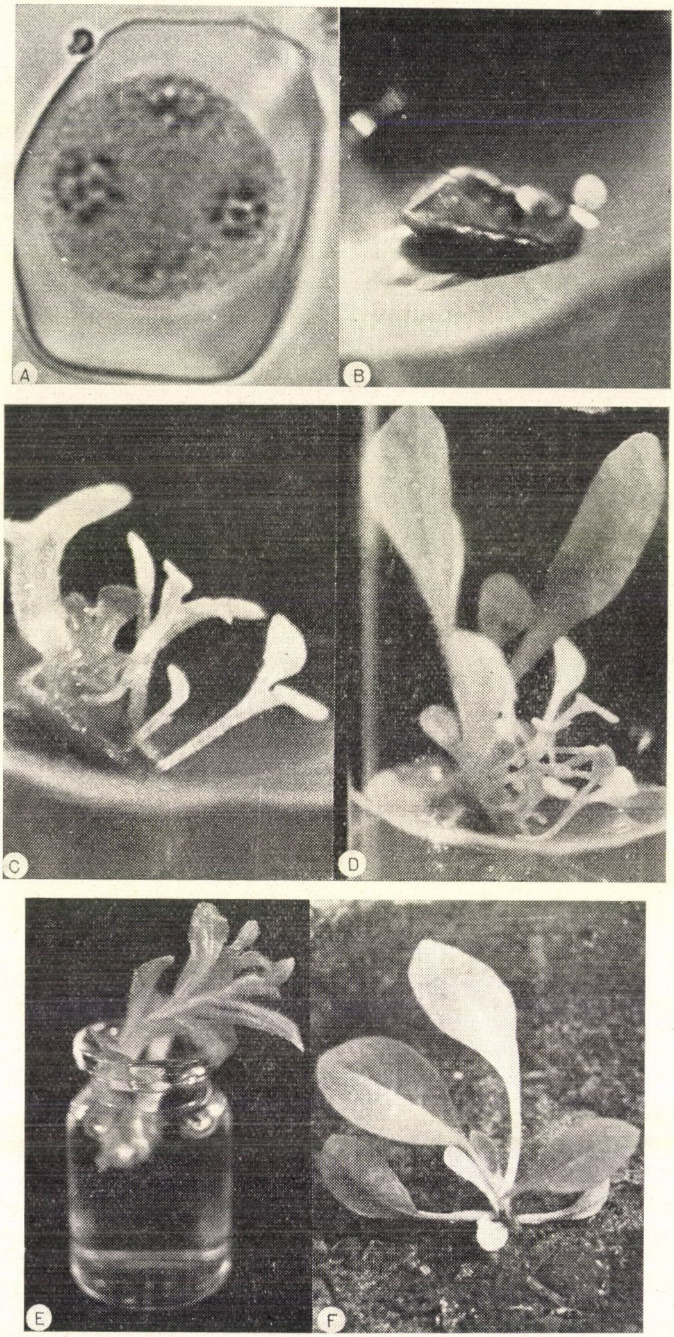

Fig. 2. Haploid plants raised from Nicotiana tabacum L. anthers isolated in Nitsch's culture media. [A. pollen mother cells of anthers isolated in the phase of sporogenesis (40 × obj., 6.3 : 1. proj.); B-C-D. haploid plants developed from anther without callus formation, at various stages of development; E. haploid plant after transplantation]

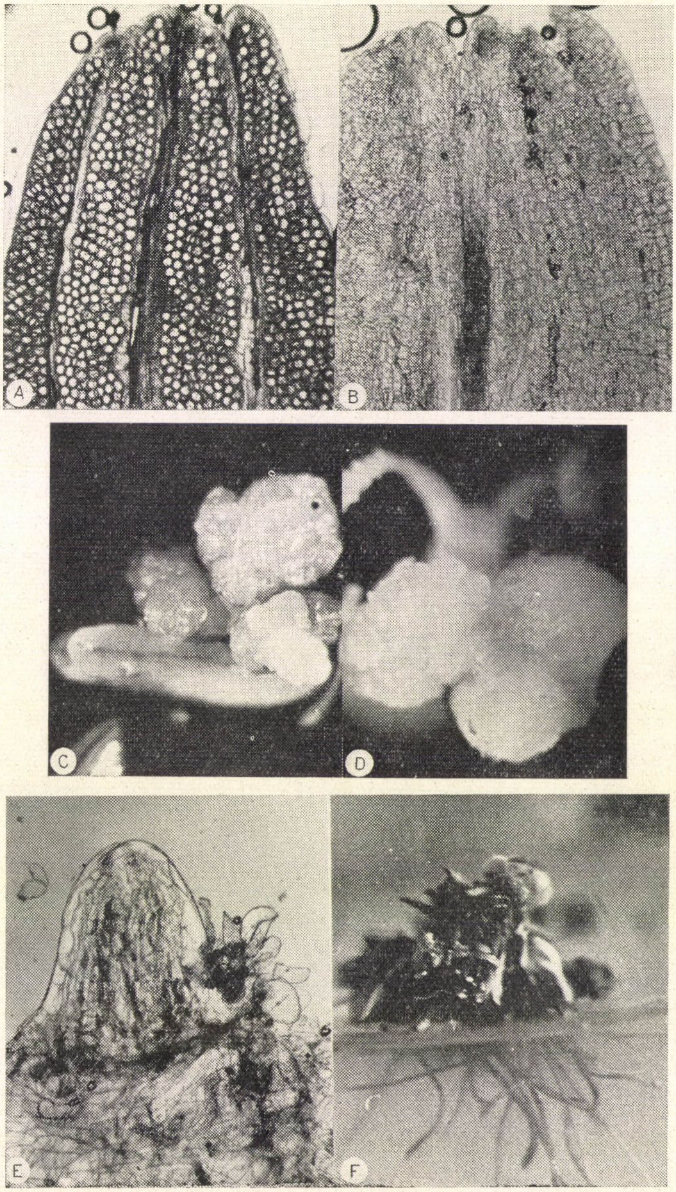

Fig. 3. Haploid callus, then root and shoot induction from *Orysa sativa* L. anthers isolated in Blaydes-culture media. [A. anthers in the phase of gametogenesis at the time of isolation (3.2 × obj., 4 : 1 proj.); B. anthers in the second week following isolation (3.2 × obj., 4 : 1 proj.); C-D. callus formation in the 4th—6th week following isolation; E-F. root organization in haploid callus isolated in a culture medium containing 0.02 ppm kinetin and 2.0 ppm IAA (40 × obj., 4 : 1 proj.)]

Fig. 4. Shoot organization in haploid callus isolated in a culture medium containing 1.75 ppm IAA and 2.15 ppm kinetin (40× obj., 6.3 : 1 proj.)

aestivum (Fujii 1970), *Hordeum vulgare* (Clapham 1971), *Lotus corniculatus* (Grant 1970), *Brassica oleracea* and *Setaria italica* (Kameya—Hinata 1970, Ban-Doi-Kokubu-Miyaji 1970 in Niizeki—Oono 1971) and of a mutant of sulphur tobacco (Burk 1970).

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A STUDY OF THE TRANSPIRATION INCREASING EFFECT OF WIND

Beside solar radiation wind is also a weather element which can be considered as a weather factor since its appearance effects the other weather elements (Bacsó 1966). This fact should first of all be taken into account during the study of the water economy of plants.

Wind substantially regulates the life processes of plants, since it not only affects the ecological factors but also influences the relationships of plants to temperature, transpiration, dissimilation and assimilation.

Senyikov (1953) attributes a great importance to the wind, since the wind changes the temperature and humidity of air and thus changes the environment of the plant. The wind increases transpiration by removing the water vapour from the stomata.

There are different values in the literature concerning the wind effect upon transpiration because in these studies the meteorological factors and the water availability by plants is variable. Wrenger (1935/36), as a result of her wind effect studies on several species, emphasizes that the various plant species reach their maximum transpiration rate at different wind speeds. The wind speed effect upon transpiration is different during the day and during the night and it also depends upon the soil moisture.

In our experiment we studied the wind effect upon the transpiration of young maize plants. The wind velocities chosen include the average wind speed of the year in Hungary, which according to Bacsó (1966) is 2.5—3.5 m/sec. The purpose of this paper is to give some data on the wind effect upon maize transpiration at different wind speeds.

The wind tunnel into which the maize plants were placed was made of wire net covered with 0.2 mm transparent PVC folia which was tightly connected to an electric fan having

Table 1

Transpiration intensity of maize plants calculated for 1 dm² of leaf-blade area at two soil moisture levels

Wind velocity m/sec	in dark		in light		Transpiration intensity of plants in light related to those in dark as a percentage
	mg/hr	%	mg/hr	%	
			40% soil moisture		
0.00	37	100	68	100	184
2.53	60	162	90	132	150
5.04	157	424	188	276	120
6.22	314	849	403	593	128
			70% soil moisture		
0.00	74	100	170	100	230
2.53	119	161	196	115	165
5.04	187	253	289	170	155
6.22	395	534	434	255	110

an output of 1 kW. The height of the semicircular wind tunnel was 50 cm and had a length of 250 cm.

The wind velocity was measured by an anemometer at heights of 15 and 30 cm at three equidistant points. Thus we made six measurements in one cross-section of the wind tunnel. The wind speed was taken as the average of these six measurements. We calculated the applied three wind speeds (2.53, 5.04 and 6.2 m/sec) in this manner at three different distances from the electric fan. Since the plants were placed in the same wind tunnel for different wind velocity treatments, measuring the two smaller wind velocities, we also took into account the speed reducing effect of the plants closer to the electric fan. The place of the two plants (replicates) obtaining the same wind speed treatment was interchanged every hour, thus the two plants were in both places for equal times during the twelve hour wind treatment. This was done to avoid any possible wind treatment difference due to the plant site in the wind tunnel.

Small, 500 cm³ plastic pots were used in the experiment, into which, for 65 dkg absolute dry soil calculated, 3.5 per cent water containing air dry alluvial-meadow soil was placed. The maximum water holding capacity of the soil was determined in a laboratory and was found to be 46.6 per cent. The soil moisture content at the start of the experiment was set for 40 and 70 per cent of the maximum water holding capacity (expressed in weight per cent) and this soil moisture level was maintained throughout the experiment by daily water supply. A uniform fertilizer application was followed in each treatment. The active ingredients of the fertilizers calculated for 100 g absolute dry soil were as follows: N: 30 mg, P_2O_5: 20 mg and K_2O: 30 mg.

For the transpiration measurements the plastic pots had tight covers which only had a hole for the stem of the plant.

The difference in pot weight between the two measurements made at the beginning and at the end of wind treatments gave the amount of water transpired by the plants.

Mv-1 maize variety was used in the experiment. Seeding time: 4. August, 1967. After emergence only one plant was left to develop in each plastic pot. The wind effect studies were conducted by exposing the plants to the air currents for a twelve hour period on 28th, 29th, 30th and 31st of August, 1967. There were two replicates for the wind treated plants and three for the wind free controls. The data obtained from the measurements of 36 plants was evaluated. In order to ensure uniformity these plants were chosen from 50 plants.

The absolute dry weight of plants and leaf-blade area were determined. The leaf-blade area was drawn on parchment paper, which was then excised and from its weight was calculated the leaf-blade surface area. The mean value of the absolute dry weight of the plants was 2.04 and 1.64 g, their leaf-blade area 5.44 and 3.41 dm^2 in accordance with the 70 and 40 per cent of the maximum water holding capacity of the soil where the plants grew.

Up to the time of the wind effect measurements the plants developed in a greenhouse. The wind treatment of plants took place in a room, where the air temperature (24.3°C) and the relative humidity of air (63.7 per cent) was almost constant. The wind treatment of plants was conducted in dark and also at low light intensity (690 lux). The light intensity, that is the light received by the plants, was measured under the PVC folia for wind treated plants as well as for wind free controls.

The data of Table 1 indicate, that the three wind speeds used in each case (two soil moisture levels, in dark and in light) increased the transpiration. But this wind effect did not increase linearly with that of the wind speed. A similar statement was made by MARTIN—CLEMENTS (1935) whose wind experiment results with sunflower plants had not shown a linear correlation between wind speed and transpiration rate either.

From the two soil moisture levels set in the experiment the transpiration intensity of the plants grown at 40 per cent maximum water holding capacity of the soil was lower for the wind free control plants as well as for the wind treated ones, than for the plants grown at a soil moisture of 70 per cent maximum water holding capacity. This applies to all of the three wind speeds used in the experiment.

Having the same soil moisture, the transpiration intensity of plants in light was significantly higher than of those in dark. This can be explained by the difference existing between the cuticular transpiration intensity and that of the stomatal one. The cuticular transpiration of well developed leaves is 10—20 times smaller than that taking place through stomata, but the cuticular transpiration of leaves upon which there is no well developed cuticle makes up half of the water transpired by the plant (SZALAI 1968). While the plants in dark transpired only through the cuticle, the plants in light transpired both through the stomata and the cuticle.

At 40 per cent soil moisture the transpiration intensity of the wind free control plants in light was 184 per cent related to those in the dark. This means that the cuticular transpiration of plants having only a slightly developed cuticle cover is 54 per cent of the total transpiration. If the same comparison is made for the plants developed at 70 per cent soil moisture, then the percentile ratio of the wind free control plants is 230, that is the cuticular transpiration is 45 per cent of the total one. This means that the transpiration rate is greater through stomata by plants developed at a proper soil moisture level, than of those developed at an inadequate soil moisture. This regularity can also be observed due to the effect of the three wind velocities with one essential difference, and this difference is that at the highest wind speed the transpiration intensity value of plants in dark and in light almost reaches one another. That is, while the percentile ratio of transpiration of wind free plants examined under dark and light conditions at a 40 per cent soil moisture level is 184 per cent, the transpiration ratio at a wind velocity of 6.22 m/sec is merely 128 per cent. An even greater percentile decrease can be observed in the case of plants developed at 70 per cent soil moisture level where this percentile ratio falls from 230 to 110 per cent.

Examining the treatments of the two soil moistures and of the two light treatments it is conspicuous that the lower the value of any wind free control the higher the percentile wind effect of the same treatment. Thus at a soil moisture of 40 per cent, in dark treatment, the transpiration intensity of wind free plants is 37 mg, but at a wind velocity of 6.22 m/sec is 314 mg. That is the greatest wind effect is 849 per cent. At the same time at a soil moisture of 70 per cent, in light, the transpiration intensity of wind free plants is 170 mg and at a 6.22 m/sec wind velocity 434 mg. Here the greatest wind effect is 255 per cent, that is much lower than in the previous case. The percentile values of the other two treatments lie between the two.

The differences in transpiration of the plants developed at two levels of soil moisture (Table 1) can be explained by the different structural make up of plants. The different soil moisture levels were also expressed by the weight and the leaf-blade area of plants. That is the weight and the leaf-blade area was smaller at the lower soil moisture. The plants with insufficient water supply underwent certain structural changes. According to WHITEHEAD (1965) if plants of similar genotype are subjected to a series of water stresses, then phenotypes which can be considered as advantageous adaptation to the conditions under the effect of which they developed will be produced. WHITEHEAD (1961) from his sunflower experiments concluded, that plants developed in wind lost less water if exposed to wind treatment than those grown in a wind free environment. He explains this water loss difference by the structural changes in plants developed in the wind. In our experiment also, very likely, the lower soil moisture brought about more xeromorph structural changes in the plants. The author (SZLOVÁK 1967) found in his earlier experiment that the evapotranspiration of plants developed in a soil with a 40 per cent maximum water holding capacity was always lower than of those plants which grew in a soil of 70 per cent maximum water holding capacity. This difference in evapotranspiration was true for the wind free control plants as well as for the plants exposed to the two wind velocities used. Since, then the wind experiment was conducted with older plants, the xeromorph character of plants, grown at a 40 per cent soil moisture level, was more pronounced than at this time.

SATOO (1953) studied the wind effect on young *Cryptomeria japonica* plants grown on three soil moisture levels and also concluded that the plants developed at a higher soil moisture level transpired more than those developed on a lower soil moisture level.

Several investigators found that the maximum transpiration of plants is achieved by different wind velocities. In GIDDINGS (1914) experiment the wind increased the transpiration of *Silphium laciniatum* L. up to a wind speed of 3.55 m/sec. A higher wind velocity decreased the transpiration rate. STALFELT (1932b) observed that the most intensive transpiration of Betula pubescens leaves occurred at a wind velocity of 1.8–2.0 m/sec. The maximum transpiration rate of the sunflower plant was observed at a wind velocity of 4 m/sec (WRENGER 1935/36). The highest wind speed (6.22 m/sec) in our experiment still increased the transpiration rate of maize. This may be due to the small plant size which was able to secure the transport of the water lost by transpiration.

Comparing the transpiration intensity at the applied highest wind speed of the four treatments (two soil moistures in dark and in light) it is clear that the obtained values are much closer to one another than can be observed in the case of the four appropriate controls. The absolute value of transpiration at 6.22 m/sec wind speed was probably determined by water uptake and water transport. While the plants at the lower soil moisture level were able to increase their water uptake and transport at higher wind speeds, at the higher soil moisture level at the given light intensity they were not able to do so. The high transpiration rate of plants with the higher soil moisture in dark was secured by the cuticular transpiration through the still poorly developed cuticle.

According to the experimental results the applied three wind velocities always increased the transpiration of maize plants related to the appropriate wind free controls at the two soil moisture levels in dark as well as in light. In each treatment the greatest wind effect was obtained at the highest wind speed (6.22 m/sec) used in the experiment.

The smaller the transpiration rate of any control of any appropriate treatment the greater was the percentile wind effect. Thus the greatest percentile wind effect upon maize plants was obtained at the lower soil moisture level in dark. Though the transpiration intensity of wind treated plants (6.22 m/sec) grown at a higher soil moisture in light was the highest, the percentile wind effect was the lowest, since the transpiration intensity of its own control was the highest among the treatments used.

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The author wishes to thank Prof. N. Bacsó for reading the final manuscript and making some useful suggestions.

*

Prepared at the Research Institute for Irrigation, Szarvas

S. Szlovák

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"ÚJMAJORI SÁRGA" PEA

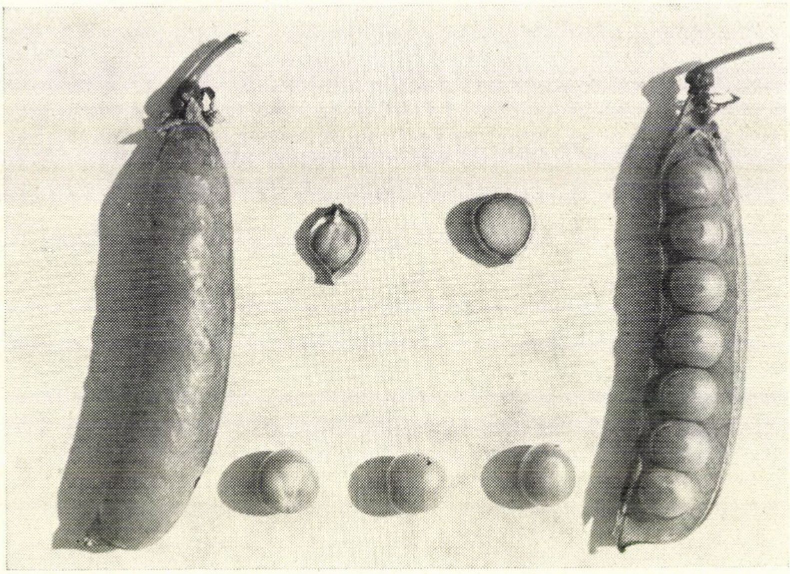
Taxonomic place: *Pisum sativum* L. convar. *vulgare* Alef. var. *superfluens* Alef.

Origin: bred by individual selection from a commercial lot

Beginning of breeding: 1947, Újmajor

Breeder: Antal Ács dr., Debrecen

State qualification: Provisionally certified improved variety, 1956; State certified variety, 1967



General characterization: a fodder pea of long vegetative period, dense foliage giving a large volume of green mass whereby it is often grown in fodder mixtures (green maize, sunflower, etc.)

Morphological description:

Root system: penetrates 100—110 cm deep into the soil and develops many root-nodules

Shoot system: 120—130 cm long shoots moderately branching, with dense foliage, though the leaves are loosely distributed on the shoots

Stem: usually divided into 14—15 internodes, angled, smooth, waxy

Foliage: leaves are large, often with 3 pairs of leaflets and a strong tendril at the apex. Leaflets mostly are ellyptic and broad; their tips are blunt and hairy; edges are unbroken, colour yellowish green. Stipules are medium spotted

Flowers: often in pairs on the floral axis (two-flower clusters), are medium large. The colour of the corolla is white. The standard is smooth edged, with a V-shaped base, its tip ends in a medium large, sharp tooth in the apical indentation. Wing blades are heart-shaped. Calyx lobes are broad-lanceolate

Pod: straight, sometimes slightly curved. Colour light yellowish green, tip blunt; 5—6 cm long, relatively narrow. 4—7 seeds develop in the pod (12—42 seeds per plant)

Seed: medium large, thousand-grain-weight 280—310 g; its shape is spherical, colour light yellow or yellow, surface smooth. Cotyledons are yellow coloured (KAPÁS *et al.* 1965)

Biological characters:

Germination: generally good, but seeds affected by drought give insufficient germination results (IVÁNYI 1966)

Vegetation period: long, about 100 days; flowering 57—60 days after sowing (IVÁNYI 1966)

Development: rapid and vigorous

Resistance to disease: when grown for seed production often attacked by powdery mildew, otherwise it is a fairly resistant pea variety

Farm technology requirements:

Seeding: in the first half of April, as early as possible, to a depth of 6—8 cm

Soil requirements: nothing particular; in good soils gives high yields

Productivity: able to yield a large volume of green mass, especially when sown with green maize; straw yield: 18—20 q/cad. yoke (1 cad. yoke = 5754, 56 m^2) Seed production is generally low, about 9 q/cad. yoke

Area of cultivation: any site in Hungary

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Prepared at the University of Agrarian Sciences, Department of Botany, Debrecen

Gy. Mándy

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Acta Agronomica Academiae Scientiarum Hungaricae, Tomus 22 (1—2), pp. 249—282 (1973)

FORUM

COMPATIBILITY OF FUNGICIDE TREATMENT AND RHIZOBIUM INOCULATION OF VETCH SEED

The study of plant protective agents (fungicides, herbicides, insecticides) has an increasing importance not only from the aspects of microbiology, plant physiology or agronomy, but from the point of view of the whole living world.

For this reason the effect of the pesticides (fungicides) on the N-fixing rhizobia and their symbiosis with legume plants, is a rather long and much studied question of soil microbiology. Inspite of this the literature (cited below) and our observations (fungicides: Kecskés—Vincent 1969a, 1969b, 1969c; Kecskés 1970a; Kecskés—Vincent 1973; Elek—Kecskés 1970; Kecskés—Szücs 1973. herbicides: Kecskés 1970b, Borbély—Kecskés 1970, Kecskés—Borbély—Borbély—Elek 1971, Borbély—Borbély—Elek—Kecskés 1972, Kecskés—Elek—Borbély—Borbély 1972, Kecskés—Nagy—Kecskés—Kovács 1972. Insecticides: Kecskés—Balázs 1972) during nearly one decade equally convinced us that the old problems as well as new important problems arise in this field day after day.

As regards this present work our aim in trying to answer the question of the compatibility of rhizobium inoculation of vetch and fungicide treatment was to find the most compatible fungicide.

In the course of studying the relevant literature it appeared that there is a great insufficiency as regards a summary or review of such literature (Erdman 1944). Because of this we give a detailed list of papers found by us on this subject from 1926 until the present.

On the basis of the literature on this subject we can distinguish the periods I. 1926—1941, II. 1941—1958, III. 1958—until the present. Naturally these periods show a close connection with the rate and need of usage of fungicides in agricultural practice.

Before the initial period of course we can find references dealing with the effect of disinfectants on the rhizobia; however, the research on this question began with the wider application of the seed protective materials: Müller—Stapp 1926, Duggar 1935, Buchholz 1936, Kadow—Allison—Anderson 1937, Litinski 1937.

It was the second period, which went into this question with greater force, but often with contradictions: Appleman 1941, Burton—Erdman 1941, McNew 1941, McNew—Hofer 1942, Sharvelle—Young—Shema 1942, Albrecht 1944, Allison—Torrie 1944, Baur 1944, Allington—Kent—Tervet—Köhler 1945, Miller 1945, Milthorpe 1945, Kernkamp 1948, Vlitos—Preston 1949a, b, Ruhloff—Burton 1951.

After a period of stagnation in the early fifties because of practical necessity the question was taken up again in different places from 1958 onwards: Hofer 1958, Hofer—Crosier 1962 (USA), Williams—Harwood—Hills (1960 (USA), Bjälfve 1960 (Sweden), Wells 1960 (Nigeria), Jakubisiak—Golebiowska 1963, Wrobel 1963, Golebiowska 1965, Golebiowska—Kaszubiak 1965 (Poland), Brackel 1963 (Belgium), Latch—Greenwood 1964 (New-Zealand), Hamed 1965 (UAR), van Schreven 1967 (Netherlands), Afifi—Moharram—Hamdi—Abd-El-Malek 1969 (UAR).

In this study the effect of six organic and one inorganic fungicide belonging to five different groups was investigated on seven strains of *Rhizobium leguminosarum* sp. Frank and their nodulation with *Vicia sativa* L.

Laboratory, light room and glasshouse experiments were carried out in the Microbiology Department of the Agricultural Faculty of the University of Sydney.

The field experiment was conducted in the gently undulating hill of the adjacent farm of the Agricultural Research Station of Wollongbar New South Wales Australia on a red basaltic or "Big Scrub" soil representing more than 200.000 acres of the Richmond River district (CRAFTS—JENKINS 1957). The test plant was *Vicia sativa* and its seed was treated with Ceresan, Spergon, Thiram inoculated with commercial peat inoculum (Root-nodule Pty Ltd., Sydney, Australia).

Fungicide treated + inoculated, fungicide treated + uninoculated, fungicide treated and uninoculated as well as uninoculated untreated vetch seeds were sown immediately, 6 hours and 12 hours after inoculation applying Amm-super Lime fertilizer.

The evaluation was carried out on the basis of the dry top weight, the nodulation, the nodulation forms, the length of top and root.

As regards the applied methods and materials most of them have been published already, details can be found in KECSKÉS—VINCENT (1969a, 1969b, 1969c), KECSKÉS—VINCENT 1973, DATE—VINCENT (1962), LEONARD (1943, 1944), PURCHASE—VINCENT (1949) and VINCENT (1970), VINCENT—WATERS (1957).

Considering the literature and the need of agricultural practice and last but not least the theoretical importance of the question we started our work.

1. Inhibition zone on seeded agar plates. *(a)* With fungicide treated seed. In a preliminary trial, seed of vetch treated with the seven fungicides according to the manufacturers' directions was placed on plates of yeast extract mannitol agar that had been seeded with *Rhizobium leguminosarum* (Table 1, line 1). All fungicides were in some degree inhibitory: Panogen the most, Phygon and Spergon the least. However, the agreement between replicates was not good, probably due to inequalities in area of contact between different seeds and agar. Filter paper discs carrying excess fungicide were substituted as a more reproducible simulation of the treated seed in the later experiments recorded in Table 1.

Seed which had been treated seven months earlier was as inhibitory as the one-month old seed reported in Table 1, in the case of 6 of the fungicides, but the activity of Panogen had been considerably reduced during this longer period of storage. A more detailed check showed in fact that the inhibition zone with this fungicide was markedly reduced even after as little as two weeks storage of the Panogen-treated seed, whether held in the light or in the dark:

Time after treatment of seed with Panogen	Conditions of storage	Inhibition zone (mm)
(a) 0 hour		48
(b) 2 weeks	dark	35
(c) 2 weeks	diffuse daylight	29
(d) 2 weeks	fluorescent light	28
(e) 40 weeks	dark	11

All differences except those between *(c)* and *(d)* were significant and show that this fungicide is unstable, rather more so when illuminated.

(b) With impregnated filter discs. The three experiments: with 7 replicate strains of *Rhizobium leguminosarum* [lines (2) and (3) of Table 1] and with 10 replicate discs tested

Table 1

Inhibition zones with Rhizobium leguminosarum and Thanetophorus cucumeris due to seven fungicides

Nature of test	Panogen		Ceresan		Cuprox		Thiram		Captan		Phygon		Spergon	
	Inhibition mm	Sig. index*	Inhibition mm	Sig. index	Inhibition mm	Sig. index	Inhibition mm	Sig. index	Inhibition mm	Sig. index	Inhibition mm	Sig. index	Inhibition mm	Sig. L.S.D. index
(1) Treated seed; *Rhizobium*, 7 strains	24.8	6	14.9	5	9.0	2	8.0— 9.4	2	7.9— 9.7	2	2.7	0	4.2	0
Filter discs: *Rhizobium*, 7 strains														
(2) (a)	90	6	51	5	46.5	4	32	2	31.5	2	27	1	22	4.3 0
(3) (b)	88.4	6	43.2	5	30.5	2	34.3	2	28.0	1	22.7	0	22.1	6.0 0
(4) Single strain, 10 reps.	110.0	6	43.3	5	29.8	2	30.5	2	26.8	1	22.0	0	21.3	5.0 0
(5) Index score (1—4)**		6		5		2.5		2		1.5		0.2		0
Filter discs:														
(6) *Thanetophorus*	149.0	6	114.0	5	27.5	0	88.0	4	51.0	3	44.5	2	35,0	6.0 1
(7)	152.5	6	111.7	5	35.5	0	86.9	4	53.1	2	46.2	2	30.7	9.4 0
(8) Signif. score (6, 7)**		6		5		0		4		2.5		2		0.5
(9) Toxicity ratio***	0.59/ 0.72/	0.65	0.38/ 0.39/	0.38	1.1 / 0.84/	0.97	0.39/ 0.35/	0.37	0.55/ 0.51/	0.53	0.51 0.48/	0.49	0.63/ 0.69/	/0.66

* Significance index = number of the 7 fungicides significantly exceeded in potency.

** Index score = Average of significance indices in individual tests.

*** Toxicity ratio = inhibition zone Rhizobium—inhibition zone Thanetophorus.

against a single strain [line (4)] showed consistent and large differences between fungicides. Panogen was the most toxic, followed by the other mercurial, Ceresan, an intermediate group of approximately equal potency (Cuprox, Thiram and Captan) and, again the least potent, the quinones: Phygon and Spergon.

Similar tests with the representative seedling pathogen *(Thanetophorus cucumeris)* followed the same general order, with the exception of copper oxychloride which was the least toxic of the seven compounds (Table 1, lines 6—8).

Toxicity ratios (Table 1, line 9: Inhibition zone of *Rhizobium* + Inhibition zone of *Thanetophorus*) show that Ceresan and Thiram were relatively less toxic towards the rhizobia than towards the pathogen. Cuprox (copper oxychloride) was at the other end of the scale.

When saturated solutions of the solid fungicides (Panogen excluded) were substituted for their use in excess, with the filter disc. method, it was found that only those of Ceresan and Cuprox were still inhibitory. The toxic effect of Ceresan could be detected at 1/100 saturation. Similarly it was only in the case of these two fungicides (and Panogen) that the substitution of the saturated solution for water in making up the medium (sterilized by filtration) prevented the growth of rhizobia. In the case of Phygon growth of 4 of the 7 strains was delayed but not prevented.

2. Influence of fungicide on the survival of rhizobia on the inoculated seed. The number of rhizobia surviving on the variously treated seed that had been inoculated with a peat culture of *R. leguminosarum* used in nodulation experiments in sand and soil (from Sydney, Narrabri, Wollongbar) was determined immediately after application and after a further 12 and 24 hours. The results of two such determinations stated in each case as a percentage of the number of rhizobia on inoculated non-fungicide treated seed, are summarized in Table 2.

Survival in the case of the Thiram-treated was uniformly good, that with Ceresan, very poor. The remaining fungicides were intermediate in effect, and could not be distinguished

Table 2

Percentage survival of Rhizobia on fungicide-treated seed

Time of exposure hr.	Fungicide						
	Panogen	Ceresan	Cuprox	Thiram	Captan	Phygon	Spergon
Rhizobium 0 (i)	57	10	42	98	72	42	72
(ii)	44	3	42	89	66	51	56
Av.	51	7	42	94	69	57	64
12 (i)	51	6	21	71	40	40	36
(ii)	62	2	74	88	86	76	56
Av.	57	4	48	80	63	58	46
24 (i)	45	<1	23	68	29	11	31
(ii)	73	3	86	110	103	82	72
Av.	59	<2	55	89	66	47	52
All times, Av. (i)	51	5	29	79	47	31	46
All times, Av. (ii)	60	3	67	96	85	70	61
Total Av.	56	4	48	88	66	51	54
Total Bacteria 0, 12, 24 hr	68	42	65	103	69	67	86

Order of decreasing survival:*

Rhizobium	T	C	P	Sp	Py	Co	Ce
	88	*66*	*56*	*54*	*51*	*48*	4
Total	T	Sp	C	P	Py	Co	Ce
	103	*86*	*69*	*68*	*67*	*65*	42

* Values underlined, not significantly different from each other.

when an account was taken of performance in both experiments. In one experiment the much larger number of "total" bacteria was also determined (viz; all colonies formed on yeast mannitol agar) and similarly compared with the non-fungicide treated control. All fungicides, except Thiram (Spergon bordering on significance) reduced the total viable count. Ceresan was again the most toxic but not as markedly as with rhizobia. The remaining fungicides were intermediate in effect and indistinguishable.

3. Indications from laboratory tests. To this stage, it could be concluded that the two mercurial fungicides were very toxic to the rhizobia and that, in the case of Ceresan, this was associated with a strong lethal effect. Copper oxychloride was moderately inhibitory and lethal and revealed a particularly unfavourable relative toxicity with the rhizobia. Of the remaining, organic fungicides which were intermediate in their inhibitory property, Thiram possessed the lowest toxicity ratio towards the rhizobia and, most important, appeared to be relatively non lethal to the bacteria applied to the treated seed as inoculum. It could be expected that on these accounts Thiram would be the seed dust likely recommended but the definite decision in this respect had to await the nodulation tests that follow.

4. Nodulation of Vetch Plants on Seedling Agar. Plants raised from inoculated fungicide and non-fungicide treated seed on seedling agar were examined for presence or absence of nodules at a stage when there had been ample time for nodulation to occur. The pooled results of the experiments spread over a period of six months are summarized in Table 3

Table 3

Proportion of nodulated plants from inoculated vetch seed according to fungicide treatment

Fungicide	Growth in seedling agar		
	Number of plants examined	Number of plants nodulated	Percentage nodulated
Nil	91	91	100
Panogen	93	50	54
Ceresan	91	0	0
Cuprox	92	30	33
Thiram	90	86	96
Captan	92	38	41
Phygon	91	38	42
Spergon	92	76	82

Table 4

Depression of nodulation by fungicide treatment (washed river sand + nutrient solution in bottle jar assembly)

Fungicide	Proportion of non-nodulated plants (out of 10)*	
	Experiment 1	Experiment 2
Nil	0	0
Panogen	0	6
Ceresan	7	0
Cuprox	0	1
Thiram	0	1
Captan	0	3
Phygon	5	5
Spergon	5	5

* Actual plants observed ranged from 9—16.

Under these conditions Ceresan completely prevented nodulation; Thiram was almost without effect and the Spergon treated seedlings were generally well nodulated. The other fungicides except Cuprox were intermediate in effect, whereas in the case of Panogen seed that had been treated 14 weeks or more before inoculation yielded more positive plants (81%) than those that had been tested earlier (9%). This appeared to be related to the instability of this fungicide already noted in connection with the laboratory tests.

5. Nodulation in Washed River Sand (Bottle Jar Assembly). Two preliminary experiments were conducted with the seven fungicides, the inoculated seed being sown 3—4 hours after inoculation. Plants were harvested in the sixth week and examined for presence or absence of nodules (Table 4). Phygon and Spergon depressed nodulation on both occasions; Panogen and Ceresan had a marked effect on one of the two occasions; Cuprox and Thiram appeared to be the least inhibitory.

In a more detailed experiment with the same assembly, the distribution as well as the presence or absence of nodules was recorded (Table 5). Under these conditions the nodulation pattern of the non-fungicide treated inoculated control was indicative of early invasion (48% nodulation near the crown of the tap root; 81% with one form of tap-root nodulation). The extreme condition of inhibition with Ceresan (31% without nodules and tap-root crown nodulation completely prevented; total tap-root nodulation 20%; distal lateral nodulation) was indicative of considerable delay — 46%. Cuprox showed a similar trend but less markedly. Thiram and Captan were clearly the least inhibitory; Spergon, Phygon and Panogen were intermediate, in that order of increasing toxicity.

From this experiment the fungicides could be classified in order of decreasing compatibility:

Captan, Thiram > Spergon > Phygon > Panogen > Cuprox > Ceresan.

6. Nodulation in *Rhizobium*-deficient Soils: Glasshouse Experiments. Undisturbed cores of soil brought from three field sites to the glasshouse were used to test the influence of the fungicides on nodulation under conditions that bore some relationship to practical condi-

Table 5

Influence of fungicides on pattern and incidence of nodulation
(Washed river sand + nutrient solution in bottle jar assembly)

<table>
<tr><th rowspan="2">Fungicide</th><th colspan="6">Percentage of plants* with nodulation</th></tr>
<tr><th>TC</th><th>TD</th><th>TDL</th><th>LC</th><th>LD</th><th>Nil</th></tr>
<tr><td rowspan="2">Nil</td><td>48</td><td>18</td><td>15</td><td>17</td><td>2</td><td></td></tr>
<tr><td colspan="3">81</td><td colspan="2">19</td><td>0</td></tr>
<tr><td rowspan="2">Panogen</td><td>10</td><td>17</td><td>23</td><td>33</td><td>15</td><td></td></tr>
<tr><td colspan="3">50</td><td colspan="2">48</td><td>2</td></tr>
<tr><td rowspan="2">Ceresan</td><td>0</td><td>17</td><td>3</td><td>3</td><td>46</td><td></td></tr>
<tr><td colspan="3">20</td><td colspan="2">49</td><td>31</td></tr>
<tr><td rowspan="2">Cuprox</td><td>11</td><td>31</td><td>11</td><td>10</td><td>11</td><td></td></tr>
<tr><td colspan="3">53</td><td colspan="2">21</td><td>26</td></tr>
<tr><td rowspan="2">Thiram</td><td>23</td><td>32</td><td>15</td><td>22</td><td>8</td><td></td></tr>
<tr><td colspan="3">70</td><td colspan="2">30</td><td>0</td></tr>
<tr><td rowspan="2">Captan</td><td>24</td><td>37</td><td>19</td><td>12</td><td>8</td><td></td></tr>
<tr><td colspan="3">80</td><td colspan="2">20</td><td>0</td></tr>
<tr><td rowspan="2">Phygon</td><td>12</td><td>21</td><td>19</td><td>36</td><td>12</td><td></td></tr>
<tr><td colspan="3">52</td><td colspan="2">48</td><td>0</td></tr>
<tr><td rowspan="2">Spergon</td><td>17</td><td>37</td><td>13</td><td>23</td><td>10</td><td></td></tr>
<tr><td colspan="3">67</td><td colspan="2">33</td><td>0</td></tr>
</table>

* Based on 57—60 plants in each case.
TC = on tap-root, close to crown
TD = on tap-root, distal
TDL = on tap-root, distal and lateral roots
LC = on lateral roots only, near crown
LD = on lateral roots only, distal
0 = no nodule

tions. Seed which had been treated with the range of fungicides and inoculated with a standard peat culture was sown on three occasions after inoculation (0, 6 and 24 hrs), and kept in the glasshouse. After 6 weeks, by which time all of the inoculated control plants were well nodulated, all plants were carefully removed and evaluated for the occurrence and distribution of nodules.

Although nodulation was classified into all of the 6 categories reported for plants grown in sand culture (Table 5), the data have been aggregated into 4 for the present purpose by combining all cases where the plants were nodulated on the tap root. The two forms of lateral-root nodulation have been kept separate because they appear to relate to differential inhibition by different fungicides (Table 6).

In all three soils the inoculated non-fungicide treated control plants showed evidence of early nodulation (78—85% on the tap root, the remainder on lateral roots near the crown). All uninoculated controls were free of nodules. Under these conditions all of the fungicides, except Thiram, departed markedly from the norm in that the proportion of early tap-root nodulated plants was markedly less than the non-fungicide control:

Fungicide	% Plants nodulated on tap-root (Average of 3 soils)
Nil	81
Panogen	3
Ceresan	2
Cuprox	5
Thiram	63
Captan	13
Phygon	4
Spergon	5

Captan, although markedly inferior to Thiram in these tests, was significantly better than the most drastic of the fungicides; Panogen and Ceresan.

Non of the fungicide-treated plants failed to nodulate completely on the Sydney soil, all showed some cases of non-nodulated plants in the other two soils:

Fungicide	% Plants without nodules	
	Lismore soil	Narrabri soil
Panogen	19	15
Ceresan	24	0
Cuprox	0	6
Thiram	0	6
Captan	2	0
Phygon	6	6
Spergon	9	7

Taking the category LD as indicative of considerable delay in nodulation we find that Ceresan (52% of total plants), Phygon (43%) and Spergon (39%) are particularly represented in this class.

A smaller soil core experiment was also undertaken with three of the fungicides used on seed sown into the Lismore soil without the use of fertilizer and thus a degree more drastic. Under these conditions Ceresan proved very toxic and there was a marked delay in the nodulation of Spergon-treated seed. Thiram again showed a considerable advantage:

A number of nodulated plants developed from fungicide treated and inoculated seeds (12 seeds were sown in 4 cores without fertilizer in Lismore soil):

Sowing time after inoculation	Fungicide			
	Ceresan	Spergon	Thiram	Control ♀
immediately	$\underline{7}$ (1)	6	6 (2)	9 (6)
6 hours	$\underline{8}$	8	10 (6)	8 (5)
24 hours	$\underline{6}$ (1)	$\underline{8}$ (2)	8 (4)	8 (6)
		22 (2)	24 (12)	25 (17)

+ = inoculated seed
( ) = tap root nodulation
__ = plants without nodules

Table 6

Influence of fungicides on pattern and incidence of nodulation (soil cores in glasshouse)

Fungicide	Sydney soil nodulation* T	LC	LD	O	Lismore soil nodulation* T	LC	LD	O	Narrabri soil nodulation* T	LC	LD	O
Nil	85	15	0	0	78	22	0	0	81	19	0	0
Panogen	0	89	11	0	10	36	35	19	0	55	30	15
Ceresan	5	37	58	0	0	19	57	24	0	60	40	0
Cuprox	5	74	21	0	11	62	27	0	0	69	25	6
Thiram	75	0	25	0	64	36	0	0	50	0	44	6
Captan	6	88	6	0	17	50	30	2	17	83	0	0
Phygon	5	60	35	0	6	42	46	6	0	47	47	6
Spergon	10	65	25	0	4	28	59	9	0	60	33	7

* Percentage of plants with nodulation:
T = on tap-root
LC = on lateral roots only, near crown
LD = on lateral roots only, distal

Table 7

Influence of fungicides on pattern and incidence of nodulation (Lismore soil)

Treatment	Percentage plants with nodulation T	LC	LD	O
Non-fungicide control	68	32	0	0
Thiram	50	38	8	4
Ceresan	5	0	0	95
Spergon	9	50	41	0

7. Field Trial (a) Nodulation. The presence of nodules and their location on the plants are summarized in Table 8 for a total of 240 plants (for each fungicide-inoculum treatment) on the one occasion of sampling. The results agree quite well with the glasshouse trial using cores of the same soil (Table 6 and 7); the proportion of non-nodulated plants with the Ceresan treatment being much less in the field experiment, however, although more than half the plants were slow to nodulate.

There were a few nodulated plants in the uninoculated controls at the first sampling but these were all restricted to the distal lateral roots.

A later sampling is included with the earlier in Table 9, in which the separate results for seed sown immediately and after 6 or 24 hrs are also shown. This Table deals only with the proportion of crown nodulated plants which is a good indicator of speedy nodulation, and avoids any confusion with late nodulated uninoculated plants of which about 30% had become distally nodulated by the time of the second sampling. The effect of fungicide was still apparent with this uninoculated material:

Per cent nodulated plants* in uninoculated controls		
Fungicide	July	September
Nil	8	50
Thiram	3	48
Ceresan	4	6
Spergon	6	21

* Nodules on distal lateral roots only

The agreement between the occasions of sampling was good and no trend could be detected with up to a day's holding after inoculation.

The superiority of Thiram and the incompatibility of Ceresan and Spergon are clearly shown on both presentations (Tables 8 and 9).

The plants that had been inoculated and were nodulated were also classified as to whether the number of nodules was abundant (50/plant), moderate (50-10) or sparse (10). This showed that when nodulation had been delayed, as indicated by the location of nodules on the distal lateral roots only, the number of nodules was also reduced (Table 10a).

(b) Yield. Top dry weight data are also summarized for the fungicide treatments, inoculated and uninoculated, on the two occasions of sampling (Table 9a). Detail of results for the three times of sowing (0, 6 and 24 hrs after inoculation) have been omitted because, although significant differences were obtained, they revealed no trend with time (Table 9b).

The order of superiority of treatments judged by the percentage of crown nodulated plants (Thiram ≫ Nil Fungicide ≫ Spergon > Ceresan) is reflected very precisely in the respective yields. The basis of this relationship is set out more analytically for each of the forms of nodulation in Table 10. Plants having the tap-root nodulated carried a large number of nodules and were the largest plants (11× — 15× the weight of non-nodulated plants and almost twice the height). Plants that were nodulated on lateral roots but near the crown also had many nodules and yielded well. Distal lateral nodulation on the other hand, indicative of late and sparse infection, was distinctly inferior and in the worst situation was little better than the non-nodulated plant.

8. Conclusion. For the purpose of a quick comparison the results obtained in this investigation can be tabulated according to compatibility between the rhizobia and the fungicide (Table 11). When this is done the superiority of Thiram over all other fungicides is

Table 8

Influence of fungicides on pattern and incidence according to nodulation forms of nodulation

Fungicide	% Inoculated				% Uninoculated	
	Tap-root (TC,TD,TDL)	LC	LD	O	LD	O
Nil	61	31	7	1	7	93
Thiram	77	29	4	0	2	98
Ceresan	2	30	52	16	4	96
Spergon	6	36	50	8	6	94

Table 9

Influence of fungicides on nodulation and yield of vetch
*Field trials at Lismore**

(a) Comparison of fungicides

Fungicide treatment		Percentage of crown nodulated plants in inoculated plots**	Dry top weight (g/plant)***	
			Inoculated	Uninoculated
Nil	July	57	0.93	0.11
	Sept.	56	4.24	0.19
Thiram	July	61	1.25	0.10
	Sept.	66	5.52	0.22
Ceresan	July	2	0.37	0.09
	Sept.	2	2.26	0.16
Spergon	July	5	0.47	0.07
	Sept.	6	2.71	0.09

(b) Sowing times+ (hr. after inoculation)

0 July	35	0.69
Sept.	32	2.78
6 July	32	0.95
Sept.	34	4.94
24 July	27	0.64
Sept.	31	3.35
L.S.D. 5%, 1%		0.10 0.39
1%		0.14 0.52

* Means based on 240 plants (20 in each of 4 plots at three sowing times)

** All uninoculated controls were without crown nodules

*** L.S.D. 5% = 0.12 (July), 0.45 (Sept.)
1% = 0.16 (July), 0.60 (Sept.)

Thiram ≫ Nil ≫ Spergon > Ceresan
1% 1% approx.
5%

+ Average of all fungicide and non-fungicide treatments (which showed consistent effects).

evident. Not only has it given satisfactory results with every plant test (under conditions as different as seedling agar to those of the field), but its relative greater potency against the seedling pathogen *(Thanetophorus cucumeris)* suggests that compatibility with the rhizobia is not at the expense of its fungistatic property.

When one looks to the laboratory tests as means of predicting usefulness under practical conditions, the agar diffusion method, though able to detect some markedly incompatible

Table 10

Relationship between form of nodulation, nodule number and plant size

	Inoculated						Uninoculated	
	Tap-root nodulation			Lateral nodulation		No nodules	Lateral nodulation	No nodules
(a)	*TC*	*TD*	*TDL*	*LC*	*LD*		*LD*	
Distribution according to								
nodule number 50	82	33	55	60	4		0	
50 10	18	67	45	39	34		0	
10	0	0	0	1	62		100	
(b)								
Dry top weight (g/plant)*	1.21	1.12	1.50	0.88	0.48	0.11	0.12	0.09
		1.27		(0.21—0.75)				
(c)								
Mean length of plant top (cm)	36.2	36.5	48.5	32.0	25.5	22.0	27.6	23.2
		40.4						

* July harvest. Note that within the nodulation categories there was no relationship to fungicide treatment except in the case of LD category where the Ceresan and Spergon yielded much less than the Nil and Thiram (0.22, 0.21 comp. 0.67, 0.75).

Table 11

Comparative summary of criteria of compatibility of fungicides with Rhizobium leguminosarum

Criterion	Panogen	Ceresan	Cuprox	Thiram	Captan	Phygon	Spergon
A. Laboratory tests							
1. Agar diffusion (Table 1)	—	—	+	+	+	++	++
2. Toxicity ratio (Table 1)	+	++	—	++	+	+	+
3. Survivors on seed (Table 2)	+	—	+	++	+	+	+
B. Plant tests							
4. Seedling agar (Table 3)	+	—	+	+++	+	+	++
5. Sand culture (Table 4)	+	—	+	++	++	+	++
6. Soil cores (Table 5)	—	—	—	+++	—	—	—
7. Field trial (Tables 7—9)	n.t.	—	n.t.	+++	n.t.	n.t.	—

— = incompatible
+, ++, +++ = compatible in increasing degree

substances (e.g. Panogen and Ceresan) did quite badly with Phygon and Spergon and failed to distinguish between Thiram and the inferior Cuprox and Captan. The best correlated laboratory result was the per cent surviving rhizobia on the treated seed which clearly separated Thiram from the rest.

Plant tests neither in seedling agar, nor in sand watered with nutrient solution were by themselves in sufficient to screen off all less satisfactory chemicals. However, glasshouse trials with undisturbed soil cores were quite adequate and correlated very well with the final criterion — the field trial.

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CONTRIBUTIONS TO THE PAPER OF E. I. KOVÁCS: "THE GENETICAL| RELATIONS OF PREFORMATION AND EPIGENESIS" PUBLISHED IN THIS PERIODICAL, *21* (3—4)

HAS THE CONCEPT OF EPIGENESIS BECOME OBSOLETE?

Works of this character — in our opinion — have to be welcome, since in this respect there is a great need in the science of biology. There are theoretical physics, general chemistry, but neither theoretical nor modern general biology exist, although they are badly wanted. Therefore all activities that would promote their existence must be supported. It is considered very useful to assess from time to time the conceptual changes of terms used in the science of biology and trace them back to the classics, as in the classics of biology there are quite a lot of presentiments found which are inspiring even today. It can be stated that there are but few sciences which make as bad use of their historical inheritance as biology does. It is, thus, of great importance to throw light upon the theoretical problems, clarify the actual meaning of concepts and express them — if possible — with up-to date terms.

However, in our opinion the work in question does not fulfill these requirements and objectives. The author seems to have met the heterogeneous interpretation of the concept of epigenesis in the literature while studying other subjects rather than to intend to solve the problem by an independent systematic investigation. This may be the explanation for the author relying on a part of the relevant literature and on a few citations only. Although the authors cited are famous representatives of the science of biology, a few citations are still not sufficient to settle such an important problem. Views much rather than definitions ought to be analysed.

Both in the literature used and among the works cited there are monographs dealing specially with this question, text-books written for university students, books merely touching upon the problem, experimental works, etc. The authors are botanists, zoologists, paleontologists, etc. This wide scope makes the work more difficult and requires great circumspection. A much clearer position could be taken if treatment were limited to zoology, by separating works of embryologic and genetic relation, and restricting analysis to theoretical elaboration.

A further deficiency is the absence of an analysis of Max Hartmann's, Alfred Kühn's, Needham's, Mirsky's, Markert's views, because, for example, it would be interesting to find out why some of them did not use the term epigenesis. The genetic vocabulary written by Rieger et al., which includes the word epigenesis, also should have been taken in consideration. Clarification of the concept of epigenesis would have required, further, a study of its correlations with closely related concepts. It is here that — in our opinion — a parallel analysis of the concepts of induction, organization, self- and dependent differentiation belong.

The absence of a discussion of gene activity and inactivation — which are not even mentioned — is a further deficiency of the work. Analysis of the question of preformation is also lacking. While the author is right in considering this concept much more unambiguous than the concept of epigenesis, in the course of time it too has undergone a considerable change.

E.g. Max Clara writes in his text-book that it is the place of development rather than the organ or tissue that is preformed. Some of the citations (e.g. Goldschmidt page 2. paragraph 2.) seem quite meaningless, while others are so short and isolated from the context that one can hardly know what the author wants to say, or there is not enough background for the reader to control by himself whether he has understood the citation correctly. E.g. the last line of paragraph 2. on page 3: "... epigenetic systems ... may occur in the nucleus too". Or the last line of the last paragraph on page 4: "... even the fertilized eggcell does not contain the complete information material of the grown-up individual ...".

It is because of the deficiencies listed above that — in our opinion — the essence of the work has got lost; namely, the historical development of the concept of epigenesis is not apparent, it is not quite clear why a new definition is necessary, whether this concept must really be discarded or only concretized and restricted on the basis of new knowledge acquired. Nevertheless there would have been an opportunity to meet these requirements even on the basis of the literature cited, and the original objectives of the work too would have been attained in that way. It is not quite clear either, whether there are really differences of principle between the authors cited, or it is simply a question of approaching the concept of epigenesis from different aspects.

The main reason for the deficiences mentioned above is supposedly the intention of the author to keep to the essentials. This is a laudable aim otherwise, but the theoretical works of biology cannot be built on a few citations, further, works of this nature by all means require an appropriate extension too.

In the first paragraph of the Comments on page 4 there is a fundamental error: in the author's opinion the amount of information expresses the extent of disorder. According to text-books in general, information in the last analysis means order, and something which has no kind of system cannot contain any information. For this very reason, from a physical point of view information can be readily brought into connection with the conception of entropy in thermodynamics. In this sense we can accept that the author, wrote disorder instead of order, since the amount of information can be expressed with negative entropy. Therefore a theoretical start from the interchanged signs would not be blamable, but towards the end of the paragraph the confusion becomes evident. Namely the author writes that in the course of ontogenesis the information material has to decrease. In this case it is not a question of information content, but of an amount of information, which increases with orderliness. If the author really means information content instead of an amount of information, then his statement may be correct, but this ought to be explained more closely and the first disturbing sentence about the amount of information omitted. We have a feeling that the author is uncertain in dealing with the concepts of information theory in other places too (page 4, paragraph 2). True, he refers in the meantime to Raven. Accordingly, living creatures receive a large amount of information from the environment during morphogenesis. Then, after a casual sentence the author says that the redundance factor is higher in the adult, while later writes about the information content as being higher in the adult. In this form it is not clear. According to the usual definitions redundance is in no connection with the content or the amount of information; it is related with the reliability of information transmission. We consider a code optimum when it expresses a given amount of information with the fewest possible signals. A series of signals is redundant when it contains a higher number of signals than necessary for the same information content. The author arrives at the final conclusion that the concept of epigenesis had better be placed in a museum.

We are not convinced of the correctness of the author's opinion, as the science of biology uses hundreds of words the conceptual backgrounds of which are not quite clear. The biologists understand each other, and even an uncertain terminology makes many descriptions simpler. According to the classical interpretation epigenesis was the opposite of genesis, but in the course of time, with the development of the biological sciences the concept of epigenesis has differentiated according to the interpretation of various phenomena without the classical view becoming meaningless. We think that the concept of epigenesis has not become completely obsolete.

A concept which has lost its actuality automatically ceases when scientific progress has gone beyond it, as often seen in the history of science. We agree, however, that the concept of epigenesis must be carefully dealt with, and when using it we must define how it should be interpreted.

When all is said we think that the paper gives little. Its final conclusion, that in the living creatures there are determination and realization mechanisms, and that the two are correlated is contained in every text-book; and the statement that both are found in the unicellular organisms too while the realization mechanism is missing in the viruses is a generally known axiom.

In Hungary there is a great need for papers of this character, as they are published in such a low number that there is simply no choice. For this reason the publication of the paper may be useful for the Hungarian biologists in spite of all its deficiencies; however, the confusion about the content of information, amount of information and redundance ought to be settled. It must be made clear whether the author means the mechanism of determination or that of realization, and the following questions should be answered: what information is received by the organism from the environment and what is it used for; is there a real decrease of entropy in the course of onto- and philogenesis; and how can the entropy correlations be used with an open system of stationary stage?

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WHAT CONCEPTS SHOULD BE APPLIED IN THE EXPLORATION OF STILL UNKNOWN EVOLUTION PROCESSES?

The paper is a critical exposition with the concept of epigenesis, as it arose in the 18th century as a counter-hypothesis against the preformation theory, and is actually employed by biologists. The Author first gives a short review on the origin of the epigenesis theory. The first main part contains a compilation of definitions from which it can be seen how the concept is applied in modern biology. The author indicates at the same time how the word "epigenesis" is applied by various authors in very different correlations and with different meanings.

The second main part contains a criticizing comment by the author on the present employment of the concepts of preformation and epigenesis. He points to the fact that in the up-to-date experimental investigations of evolution two processes have been revealed: the genetical information, through which the evolution is determined and the realization mechanism, which comprises replication, transcription, translation, as well as genetical-physiological regulation mechanisms and in turn depends upon the determination mechanism. Both are operating together in the ontogenesis.

On the ground of the short but thorough and very criticizing exposition with the problems and foundations of the experimental examination of evolution the author comes to the conclusion that in consideration of the more recent knowledge, old words — which nowadays are already deprived of sense — such as epigenesis and epigenetic processes, should no longer be employed. In the exploration of still unknown evolution processes such concepts should be applied which denote exactly what should be examined, correspondingly to the present state of knowledge, namely the determination-mechanism and the realization mechanism.

The paper of Kovács is a very good contribution to the clarification of concepts which are used in working with biology. On one example he demonstrates, how not-clearly defined, historically burdened words contribute rather more to the veiling, than to an eluciditon of the problems.

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A BELATED DISCUSSION ABOUT PREFORMATION AND EPIGENESIS?

When dealing with the history of science we — naturally — take out the old authors' writings and revise the early findings. Ervin Kovács dr. too begins his work in this way, although he does not intend to revive researches carried out in the past; he keeps the claim of biocybernetics in view: to explain the concensus of genetic information and realization mechanism. I admit that it was not at once that I found out what it was all about, and there is no such statement in the paper either. It took reading the paper through several times to recognize its real purpose, and though it is only an imputation that the author looks toward cybernetics I do not believe that he would deny it. It is no question of late discussions; the point is to uncover still living anachronisms in order to see clearly. Kovács's paper activates questions of latent state, and such questions, at that, as familiar even in quite recent works; e.g. the concept of epigenesis is discussed without any comments in the reduced size 1971 edition of the Természettudományi Lexikon (Encyclopaedia of Natural Sciences) as if it were an unambiguously clarified rather than a dim term. The following quotation from Rajki et al.'s book is highly relevant here: "It is necessary to protest not so much against the phlogistonization of scientific language, as against the demand to consider this phlogistonization compulsory for all." (Metabolism and heredity . . . Martonvásár 1972; p. 39.)

Beside the problem of using clear and up-to-date terms the paper raises a long discussed question too while touching upon the genetic implications of preformation and epigenesis. One of the problems may be put like this: is the information content of the zygote identical with the content of the adult organism? I think the above cited book with the successful experiments on the autumnalization of summer wheats described in it gives an answer to this question. (Sapienti sat!)

Another periodically recurrent problem–which Ervin Kovács dr. treats casually and of which he gives his opinion only in brackets —is whether or not the virus is a living organism. Truth may have more than one side; a virus getting out of an animal or plant — more properly called now a *virion* — cannot, indeed, be a living organism, since it has no enzyme system and, consequently, no metabolism. But the *vegetative virus* becomes and remains part of the living organism until it is joined in the latter's metabolism.

The question catalyzes itself further; was the virion reduced into inorganic matter from one or another of the living systems? Can it be regarded as a bacterium which has lost its metabolism-enzyme system with only the nucleic acid representing the genetic information left in a protein cover? Or is the virus a catalyzer inorganic from the beginning, with a tendency to become a living organism?

I think science has made a concession in these questions when regarding the virus as a transition between living and dead matter.

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WHAT IS GAINED BY REPLACING THE TERM EPIGENESIS WITH OTHER TERMS?

In my opinion, the term epigenesis, denoting the theory that new structures and organisms develop from an originally undifferentiated mass of living material in the course of embryonic development, is a general term and is useful because of this. It is, therefore, not ambiguous, but rather it is useful to describe development as being the complex interaction of genetic and non-genetic processes.

It is hard to see what advantage in understanding, or clarity, is gained by substituting the term "*determinative mechanism*" for genetic system; or, for substituting the term "*realizer mechanism*" for epigenetic system. Perhaps specific experimental results might make the meaning of "*realizer mechanism*" better understood, but as it is, it seems to carry the same meaning as epigenesis. I do not see the need to add another term to make the issue more confusing.

There is nothing wrong in describing ontogenesis as an epigenetic process when this has the meaning of complex interaction of genetic and non-genetic processes. The author is of course right when he states that terms such as "epigenetic factors" do nothing to explain the unknown complexities underlying morphogenesis. However, deleting the term epigenesis and replacing it with other terms also does not further our knowledge of the processes involved in morphogenesis.

No reference is made to recent research in developmental biology; the most recent reference given is 1963.

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SHOULD BOTH EPIGENESIS AND PREFORMATION BE PUT INTO A MUSEUM?

The author, dr Kovács has a clear view on the guiding factors of development. The majority of geneticists would obviously agree with his opinion. There could be some objections, however, in two aspects. First, the literature of the last ten years is neglected in the article; second, some of the Author's reasoning seems to me not completely consistent.

Both, preformation and epigenesis are obsolete terms in their original sense. Nowadays they are regarded as naive curiosities in the history of biology. We have to admit however, that in the recent literature on embryology and developmental biology the reader often comes across scientific terms like "epigenetic system," but these are of different meaning, as dr Kovács also emphasizes, and only the words are somewhat similar. In Waddington's (and also Nanney's) opinion (as far as I can understand) the epigenetic system is a sequence of genetically controlled realizing processes influenced (or canalized) by the inner and outer environment. Dr Kovács criticizes this definition by stating it is ambiguous, because it does not make "sharp distinctions between the genetic and non-genetic processes of development." Later, under the "Commentary," having previously altered the term "epigenetic mechanism" to "realizing mechanism" dr Kovács makes exactly the same "mistake": "The realizer mechanism includes replication, transcription, genetic and physiological regulation systems." The separation would be extremely difficult, since the genetic and environmental factors are in close interrelationship, and even the external environment can be under "genetic" influence (as illustrated in Fig. 13 in Waddington: The Strategy of the Genes. — George Allen and Unwin Ltd., London 1957).

Dr Kovács recommends to put the word "epigenesis" into a museum. Provided this term means the original idea (or a modernized form of it, with basically similar concept), this proposition is obviously acceptable. It should be mentioned furthermore, that the word "preformation" is just as much obsolete, and therefore could be placed into a museum too, keeping in mind, that preformation is not quite identical with predetermination, since it presupposes the presence of the *form* (and not just the information on it) in the gametes.

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WHEN DO THE TERM DETERMINATIVE MECHANISM AND REALIZER MECHANISM BECOME SUPERFLUOUS?

E. I. Kovács discusses the usefulness of the term epigenesis in the analysis of the development of an individual organism. Epigenesis means the ordered sequential transcription and translation of genes or groups of genes as we observe it during the development of a cell, an organ or an organism. The term also covers the interaction of the genes with each other and the interplay of the genotype with the environment during development. Kovács would like to see this term abolished. I agree that terms do not solve problems. Indeed, the word epigenesis has contributed little to the elucidation of the problems of development. As a descriptive term it appears today unfortunate because — as Kovács points out — it covers too many and too diverse phenomena, phenomena which furthermore in an eukaryotic cell must be highly complex. Increasing numbers of mechanisms that control gene activity negatively or positively are being uncovered and each of these mechanisms helps us to understand some aspects of development.

Kovács proposes to describe the development of an individual organism as regulated by the determinative mechanism and the realizer mechanism. The former is limited by the genetic information (the idiotypus) available to the cell or organism at any one time. The latter comprises the conditions and the molecules necessary to express a certain genetic message. These terms become superfluous as soon as we can describe a developmental sequence by all the involved molecular steps. This goal has already been achieved in a few cases.

The morphogenesis of a T_4 phage particle as studied by Edgar and Wood is in principle completely known and no longer requires the assumption of unknown mechanisms. As soon as the DNA of the particle has entered the bacterial host, the phage genes are transcribed and translated in the order in which their products are used to assemble some hundred new phage particles. In this case the sequence is established by the linear order with which the genes are arranged on the phage genome. Certain of the produced phage components have the properties of self-assembly, others require an enzyme for their assembly. Translation of the phage messenger RNA takes place on bacterial ribosomes. This type of cooperation we are likely to encounter in eukaryotic cells as well. Many messenger RNAs transcribed from nuclear genes are translated on cytoplasmic ribosomes, others probably on chloroplast ribosomes. Some messenger RNAs transcribed from organelle DNA in turn might be translated on cytoplasmic ribosomes.

The morphogenesis of λ phage particles as analysed by Szybalski and coworkers is likewise effected by an ordered sequence of transcription, whereby genes or groups of genes are transcribed as soon as their promotor site is available for the RNA-polymerase to act. Transcription starts at one point on the chromosome but proceeds in two different directions, thus providing an extra parameter for the regulation of the developmental events. Phage λ illustrates another important aspect, namely two alternative "developmental" cycles of the phage. In one situation the phage genome (prophage) replicates as an integrated portion of the bacterial chromosome. This lysogenic cycle continues for many cell divisions; it continues as long as the λ repressor protein covers a certain gene of the phage. Alternatively the phage enters the lytic cycle resulting in the production of new phage particles and lysis of the bacterium. This route is taken when the repressor protein fails to be synthesized or when it is modified in its conformation, so that it can no longer bind to the gene in question and therefore permits its transcription.

These developmental sequences are relatively simple and therefore understandable in principle through the sequence of molecular steps now known. I believe that the complex, interdigitated developmental sequences apparent in the ontogeny of a higher organism will also be understandable, as soon as we have mapped the molecular events occurring in the eukaryotic cell. Plant cells operate with a large amount of genetic information in the nucleus, a smaller amount of genetic material in the chloroplast and a still smaller amount of DNA in mitochondria. Plant cells translate the transcribed messages on either cytoplasmic or chloroplastic or mitochondrial ribosomes. The compartment in which a given protein is synthesized is frequently not the one in which it is put to use.

It is a primary task of developmental biology to determine in a given organism for each individual protein molecule the location of its gene, the time of transcription, the site of translation as well as the cellular site of its function. To give an example: Wildman and coworkers have recently discovered that the CO_2 fixing enzyme in chloroplasts of tobacco, the ribulose-1.5-diphosphate carboxylase consists of two polypeptide chains, one of which is coded for by a nuclear gene and the other by a gene located in chloroplast DNA.

As our picture on different molecules, organelles and cells is filled in this way, we will grasp how an organism grows and develops.

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WHICH ARE THE EVENTS DETERMINING THE DEVELOPMENTAL FATE OF A CELL?

Panta rhei and the concepts of science are constantly changing. The emerging new methods open new perspectives for science which result in new concepts and models in the interpretation of scientific phenomena. In the science of development the application of better microscopy put to an end the concept of preformation (a mostly philosophical one) and created an epigenetical way of looking at developmental processes. The methodological facilities of our time have led to a molecular approach of biological events which have been adopted for analysing developmental processes.

In the following I should like to deal with the determination problem of development.

WADDINGTON (1957) in the causal analysis of epigenesis (epigenetics) concludes that the end products of development show a discontinuous variability i.e. the developing end products separate themselves in a discrete manner. There are no intermediates, if so, they are insignificant. It means that the evolving end products are determined. This is the central idea of epigenetics. The epigenetic landscape of Waddington — created as a three dimensional model for the demonstration of the developmental process — is a subject of the determining genic effects (or gene products) in a coordinated system. The coordinated genic effects create the "necessary paths" (creode). The cross section of the landscape "indicates the strength of the system to return to normality 'the intensity of homeorrhesis'" (developmental homeostasis). The emerging of the landscape is therefore determined by the genes (or gene products) and the changing of one gene (by mutation) does not cause concrete changes in the process as a consequence of the strong interaction of the different genes which decide the profile of the landscape. Therefore if a cell takes its position on some point of the starting line of the landscape — its fate is determined.

Which are the events which bring the cells on the different points of the landscape, which determine their developmental fates? This course of events is the determination process by which the cells become determined.

"Determination is among those indispensable terms in tne vocabulary of embryologists and geneticists which is most difficult to define" and "Determination is a process, which initiates a specific pathway of development by singling it out from among various possibilities for which a cellular system is competent" (HADORN 1965). The cleaving cells of an embryo become determined before differentiation starts and this is realized after accepting a signal from somewhere. The location for the formation of a puff on the chromosome is determined, the formation of this puff, however, starts after being signalled by ecdyson. During embryogenesis at one time (probably in the blastomere) the cell becomes determined and is able to transfer this stable determined state during long cell generations. Thus in the early phase the zygote has a totipotency and this is lost during the cleavage at the advent of determination.

What are the mechanisms by which the determination probably effectuates? On the level of DNA: chromosome diminution, somatic cell elimination, heterochromatization. These are gross structural changes which probably bring the cells to be determined. Other possibilities for the determination are the reversible/irreversible inactivation (repression) or activation of specific loci on the chromosome. These are the cause of the "differential release of informations" (SCHULTZ 1965). This means minor structural (functional) changes of the chromosome inducing a process which later, together with gross structural changes, appear (puffing, lampbrush) to set synthetical processes going (DNA = gene amplification of RNA genes; RNA = mRNA synthesis).

But these are changes on the level of DNA caused by determination. The questions now are: what is the time and the place, and when and where do the cells become determined in the developing embryo?

Benzer (1971) introduced the "fate mapping" of the embryo *(Drosophila)* by using the mosaic technique. The results clearly demonstrate that the position of the cells in the blastomere determines the fate of the descendant cells. That is to say the determination takes place at the time when nuclei are enclosed into distinct cells, and the membrane formation starts. Garen concludes that the determination "is not a function of the nucleus . . . The effect of mutations in the mother *(Drosophila)* fly, which is expressed only in the developing egg, implies that some pattern of information may be laid down even before fertilization." Its seems therefore that not only those informations realized during development which are programmed in the nucleus but the cytoplasm, its metabolic loops, autonomic structures, also have some effect on the determinative processes.

The subtle interaction of the genetical informations and the cytoplasmic states (microecological successions) and perhaps other programming tools of the developing embryo are the devices for determination. The diversity of the cellular events are so manifold during development that some people are hardly able to accept that every step of the developmental program is laid down in the genes (structural and regulator) of the organism. It is unlikely that the genome contains so much information for the behaviour of every cell during the developmental process. My opinion is — and I suppose I do not stand alone in that question — that in the events of development we must know precisely how they occur during determination and formulate in molecular terms what happens when a cell becomes determinate and we must know what kind of reciprocal events the whole process depends on. Until then — I am afraid — the whole question remains rather a matter of semantics.

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CAN DEVELOPMENT BE CONCEIVED AS PREFORMATION?

In his paper "The genetical relations of preformation and epigenesis", **Kovács** (1972) discusses various modern definitions of epigenesis and concludes that most of them are ambiguous. The idea of preformation is much more unequivocal than that of epigenesis. It would be desirable to put the obscure and ambiguous word "epigenesis" into a museum. Instead of a preformational and epigenetic mechanism, he prefers the terms "determinative" and "realizer" mechanism. Both mechanisms operate simultaneously in the process of growth, differentiation and organization.

Though agreeing to a large extent with these views, I should like to make some comments.

In my opinion the ambiguity of the term "epigenesis", emphasized by Kovács, is mainly due to the fact that this concept has no positive content. Through the ages, "epigenesis" always denoted the negation of preformation. Whereas the latter term pointed to some mechanism supposed to provide an explanation of the phenomena of development, "epigenesis" in itself did not indicate such a mechanism, but merely the lack of it. This holds both for the epigeneticists of the 17th and 18th centuries, repudiating the "predelineation" of the adult in either egg or sperm, and for the epigeneticists of this century, rejecting Wilhelm Roux' "predetermination" of the embryo in the fertilized egg.

The failure of the epigenetic view to provide an explanation of development is the more serious, since the whole antinomy of preformation and epigenesis is closely linked up with the problem of order and disorder in nature. It is one of our deep-rooted intuitive convictions that order does not arise "by itself" out of disorder. In physics this has led to the various formulations of the second law of thermodynamics. In biology the apparent increase in ordered multiplicity during development offers nearly unsurmountable difficulties to our understanding, since it seems to be in conflict with this prescientific conviction. Therefore, those striving after a scientific explanation of development at all times have tried to get round this difficulty by assuming that no new-formation of ordered spatial multiplicity during development took place, since the ordered structure of the adult animal somehow preexisted from the beginning (preformation). It is quite easy to repudiate and ridicule this theory. It is less easy to replace it by a better one. Anyhow, mere rejection of the preformationist view (epigenesis) offers no solution. Most epigeneticists of the 17th and 18th centuries were aware of this and had recourse to some kind of vitalistic agent, creating order out of disorder. But our modern epigeneticists in general did not follow Driesch on his road to vitalism. Rejecting both preformation and vitalism, they do not supply an alternative theory and leave us empty-handed.

In order to arrive at a satisfactory theory of development, doing justice both to the apparent facts of morphogenesis and to our conviction that order cannot arise out of nothingness, it will be necessary to start from a purified and modernized notion of preformation. This concept has been reconsidered by VON UBISCH (1942) and by myself (RAVEN 1952). We both came to the conclusion that a scientific explanation of the phenomena of development is possible, without having recourse to "vitalistic forces", if a certain amount of preformation is accepted. This modern version of preformation is distinguished from the "predelineation" of 17th century embryologists as well as from the "pre-determination" in the sense of Roux by the fact that it only demands that the ordered multiplicity of the egg cell is quantitatively equivalent to that of the embryo developing from it (pre-diversification), without any conditions made as to the localization of the extant differences. If this requirement is met, an adequate description of development in terms of physics and chemistry seems thermodinamically possible.

At the time, this requirement of quantitative equivalence of the structure of egg and embryo seemed a rather abstract postulate, since no method could be indicated to measure the amount of "ordered multiplicity" of a system. Since then, however, the rise of information theory has provided us with the possibility to measure "order" and express it in numbers. This enabled a new approach to the problems of development.

ELSASSER (1958) has the merit of first stating clearly that the theory according to which the egg contains all necessary information for its development into an adult, means a modern version of preformation theory, whereas the opposite view, according to which part of the relevant information is gained during development from other sources, can be called epigenesis. Elsasser himself decided in favour of the latter view. I, on the other hand, accepted the preformationist thesis as a starting point for applying information theory to the problems of development (RAVEN 1961).

Shannon (1948) was the first to define a useful measure of information, usable in the comparison and evaluation of communication systems. For a wider application of information theory in science, however, it is **Brillouin**'s book (1956) that is especially important.

Brillouin distinguishes between "free" information, an abstract quantity, which general information theory is dealing with, and "bound" information, relating to a physical system, in which the possible choices are interpreted as micro-states (complexions) of the system in the sense of thermodynamics. This bound information is related to the concept of entropy.

Every physical system is incompletely defined. The values of some macroscopic variables are known, but it is impossible to specify the exact position and velocity of all particles. The greater part of the information on the micro-structure of the system is lacking. Entropy is often defined as a measure of disorder in a physical system, but according to Brillouin it is more correct to consider entropy as a measure of our lack of information on the real structure. In consequence of this lack of information there are a great number of micro-states between which we cannot distinguish in practice. If more information on the system is obtained, some configurations may be precluded; the number of possible micro-states, and hence the entropy of the system, diminishes. Therefore, increase of information means decrease of entropy. Bound information is the negative of entropy (negentropy). Information can also be considered as the difference between two entropies, S_0 before information was acquired and S_1 after that, hence $I = S_0 - S_1$.

A comparison of various physical systems shows that the importance of such a system as a carrier of information increases in proportion to its negentropy. A system possesses negentropy when the distribution of its elements deviates from a random distribution, so that there are local differences in temperature, pressure, electrical potential, chemical composition or other variables; hence, when it has a spatial structure.

A great deal of confusion has arisen from the fact that the term "information" has been used differently by Shannon and Brillouin. Information according to Shannon (H) is a measure of the uncertainty in the system, hence of possible (potential) information; this has also been called "selective information" (Mackay). Brillouin, however, is dealing with acquired (or actual) information (I), also called "descriptive information". It is evident that within a system potential and actual information (H and I) are complementary.

This can be further elucidated by the following considerations made by **Gatlin** (1966). If nothing is known of a system and therefore all micro-states have an equal probability, the uncertainty is maximal ($H_{\max}$). If observations are made and hence information is acquired on the system, some states will become more, other ones less probable; therefore the uncertainty diminishes. The acquired information equals the difference between the maximum uncertainty and the uncertainty (H_{obs}) remaining after the observation: $I = H_{\max} - H_{\text{obs}}$. The probability distribution becomes maximally unequal when complete information about the state of the system has been acquired. One of the p_i's in Shannon's formula

$$H = -\sum_i p_i \log_2 p_i$$

then has become $= 1$, the others are zero, the remaining uncertainty $H_{\text{obs}} = 0$, and $I_{\max} = H_{\max}$. In other words: the maximum amount of information obtainable about a system equals the maximum amount of uncertainty existing beforehand.

It is further important for the application of information theory to material systems to bear in mind the relative nature of information measures. The calculated information content of a system depends on the choice of the elements considered as information carriers and on the number of classes distinguished. If the elements and classes selected are small, a very detailed information is obtained, but a great part of it may have no importance for the purpose. To give an example: for the estimation of the information content of a book it is not necessary to take account of the place of each particle of the printer's ink. For the application of information theory to a physical system, therefore, as a rule part of the total information ($H_{\max}$) can be left out of account as irrelevant information, either because it is not recoverable in principle or in practice, or because it is without importance for the question at issue and would only supply valueless detail. It must be decided from case to case which part of the information is relevant in the context. Of the relevant information, part is redundant as a rule. What remains after elimination of redundancy, is the specific information that characterizes the system.

Much of the confusion and equivocation in the use of the term information in biology can be ascribed to the above-mentioned causes. For instance, when authors, in a paper on the application of information theory to development, write that "the information content of an organism would be the same whether living or homogenized" (Apter – Wolpert 1965), they evidently have fallen a victim to the usual confusion of information and entropy, confounding total uncertainty ($H_{\max}$) and relevant information. The latter is conditional on the conservation of negentropy which is lost during homogenization. It seems to me that a similar confusion plays a part when Kovács (1.c) writes that "the degree of disoreder can be expressed quantitatively by the information content" and concludes that the increase in the degree of organization during ontogenesis brings about a decrease in information content.

In my book "Oogenesis" (1961) and, more extensively, in a recent book in the Dutch language (1968), I have tried to draw up a consistent theory of animal development considered as the processing of information. A brief outline of this theory will be given here.

As a first approach, we will confine our problem to the development of the species-specific structural pattern, leaving individual phenotypic differences out of consideration for the present. It is evident that this is the problem that mainly interests the embryologist: he studies the development of the chick, not of this individual hen.

It must be emphasized that the concept of "species-specific pattern", as used here, is not intended to mean some "metaphysical essence" of the species, but is taken quite pragmatically as the sum-total of its main distinguishing traits. We may then say that animals in general inherit this pattern from their parents. Though its development is only possible in a certain environment, environmental factors hardly ever are able to alter it into the pattern of a different species, although they may give rise to various modulations of the pattern.

This limitation of the problem greatly facilitates our task. It means that only information bearing upon the development of the species-specific pattern has to be taken into account, all other information being considered irrelevant.

The preformationist thesis now is that all information relevant to the development of the species-specific pattern is transmitted from parents to offspring in the form of structural particulars of the fertilized egg cell. Together they constitute a message, written in a code, which is decoded and processed during development, leading to the establishment of the species-specific pattern. Environmental factors may modulate this pattern, but do not determine its main traits; therefore, information extracted from the environment is irrelevant in this context.

Elsasser (1958) has repudiated the preformationist view. His main argument is the improbability of the assumption that the information content of the adult organism is wholly contained in the germ cell from which it develops. It is evident, however, that what Elsasser is talking about is the total information content ($H_{\max}$) of the system. That this is larger in an adult than in an egg cell of the same species is self-evident. During development a large increase in total information content occurs both by augmentation of redundancy of the message (e.g. in the repeated reduplications of DNA during cleavage) and by the uptake of irrelevant information from the environment. But this does not mean that something new is added to the inherited message: the specific information remains the same.

From the point of view of the embryologist, interested in the development of the species-specific structural plan, therefore the preformationist thesis provides a satisfactory starting point for the establishment of a consistent theory of development. However, other points of view are conceivable in which development plays a part e.g. that of the population biologist, interested in the adaptation of individuals to a varying environment, or of the psychologist, who studies mental development in the human child. It is evident that in such cases the emphasis is placed quite differently. The information extracted from the environment, which has been called irrelevant above, in this new context must be included in the relevant information, since it has, in conjunction with the factors of heredity, a determining influence on the development of the individual. Clearly, a preformationist view is out of the question here, as is most evident in mental development in man, where storage of new information in the brain may continue in principle to the end of individual life. One might therefore call this an example of "epigenesis", but we can agree with Kovács that this term only serves to conceal our ignorance. The manner in which the information transmitted by our senses is laid down in the structure of the brain is still largely unknown, but there is no reason to think that it will remain so for ever.

Evidently, whether development can be conceived as preformation or not depends on our point of view. As rightly remarked by Kovács, actually the structure of organisms is the result of the joint influence of genetical and environmental factors. Just as in all manifestations of life, we find also in ontogenesis a combination of two more or less opposite tendencies: on the one hand, the conservation and careful transmission of valuable attainments; on the other hand, the exploration and exploitation of new possibilities offered by the environment. It depends on us which model we prefer to apply to our field of study.

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IS IT RIGHT TO DIVIDE THE DETERMINING AND REALIZING MECHANISM INTO PARTS?

"*Homo sapiens sapiens*" has been aware of the existence of heredity for thousands of years. The prehistory of mankind and the myths are eloquent proofs of this fact. Since then we have been burning with curiosity to solve the mistery of heredity.

After the first naive ideas of the dim past the panspermia theory of Anaxagoras (500—428 B.C.), Hippokrates (460—370 B.C.) and Demokritos developed. This was followed by the doctrine of preformation which was built on an already much firmer factual biological knowledge. The third step, the postulation of epigenesis, is in essentials the recognition of biological processes being not as simple as so far believed.

Today, in possession of an immense biochemical and biophysical knowledge we know much more details of the regularities of inheritance and ontogenesis.

Following the demonstration of the existence of a genetic code and the partial recognition of the individual phases of ontogenesis we now know more — and that in a different way — about this process than even ten years ago. In the revolution of the science of biology the rapid development of genetic knowledge was of decisive importance. In spite of this fact we cannot say even today that the details of the mechanism of transmittance and ontogenesis are in every respect clear. On the contrary, we must admit that the picture, instead of being simpler and easier to survey, is becoming more and more complicated.

The preformists could not be right as they thought the mechanism of transmittance too simple. Still they were not on the wrong track in spite of the fact that their theories were almost exclusively built on empirism and logical sequences. Today we know that preformation really exists in the hereditary transmittance, only it is not like that imagined by Spallanzani or Leibnitz. The situation is similar with epigenesis. In a certain sense the process of ontogenesis is really epigenesis, only it is not as simple as thought by Wolf and his disciples.

The genetic code complement of copulating gametes is really preformed, but it does not consist of perfect characteristics only of their determinants. And the ontogenesis is not the simple growth of miniature embryos but a multiple process which produces the characters of the embryo from an "amorf" state. Thus the predecessors were on the right track, and it was a pity that they could not reconcile the two theories as Nanney tried to do so later.

The way E. I Kovács put it in his brief summary is, in essentials, a new categorization of preformation and epigenesis — or in Nanney's formulation genetic and epigenetic classification. Kovács attributes the process from fertilization to the completion of ontogenesis to the actions of determining and realizing mechanisms.

The new definition is appropriate, and the statement that the combination of these two mechanisms was of decisive importance in the beginning of "life" is also relevant.

The determining mechanism is, in fact, a tendency to stabilize. The realizing mechanism is the possibility of mobilization. In this sense we can accept the distinction of the two actions. Otherwise we might ask whether it is right to divide a fundamentally uniform sequence of functions supplementary and conditional upon one another into parts.

The demonstration of the existence of the DNA code and the recognition of the partial processes of transciption, translation and protein synthesis have opened a broad perspective for genetic research. In a short time the purposeful alteration of heredity may become possible. But even then we must reckon with the situation so much characteristic of biological research that with the growing amount of information the processes will become more and more complicated and more complex than supposed.

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GENETIC DETERMINATION AND VARIABILITY SIDE BY SIDE?

It is generally known that plant species, and individual plants, respectively, have some capacity for self-adaptation, as e.g. ecological pathological resistance, induced morphogenesis, etc. The appearance of characters is realized, and determination more or less modified by the state of nutrient supply and by the hormone interrelation which is influenced by the temperature. In this sense the ecological conditions indirectly influence the determined effectivity of the genetic system. The dynamic change of varieties, populations, stabilized ecotypes, etc. suggest that the genetic system has more than one possibilities of realization existing side by side, and one of them — the one that accounts for the change — has become effective. The cases of mutation and back mutation — especially when starting from a single variety — have brought forth numerous (nearly all) types, or new, so far unknown forms of the variety circles. On the basis of experimental inductions we may suggest that besides the ecotypes existing as components of the flora the individual contains in a "latent" though "determined" form numerous (innumerable?) genetic systems of known cultivated varieties and unknown mutant types as a model of the epigenesis, which in the cases of back mutation is an especially conspicuous phenomenon. Although spontaneous and induced mutations as changes in the DNA structure are not yet known, from the point of view of molecular genetics an analogy has to be assumed on the basis of the data of microbial genetics. Determination originating from the unity of structure and function in the biological organisms can supposedly be related with three essential stages: 1) during the ontogenesis, besides the stability of the genetic system, morphogenetic changes that modify the realization of characteristics to a considerable extent; 2) mutations and back mutations inducing genetic changes, theoretically in a reversible form and within the limits of the variety; 3) macromutation changes of phylogenetic nature which cause significant qualitative differences, are irreversible and cannot be corrected with back mutation. In the course of the ontogenesis the three stages mutually overlap — or may overlap — each other in the plants, as a very consequence of endogenous induction controlled by the hormone interrelation.

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WHY DOES THE ORGAN-FORMATION PRECEDE THE TISSUE DIFFERENTIATION IN THE COURSE OF THE EMBRYO DEVELOPMENT?

With a one-celled organism which performs all functions taken as a basis the information can be considered as continuous, where one phase follows another at certain intervals: reproduction, growth, differentiation and again reproduction. In the meantime motion, nutrition, life processes take place. In a multicellular organism the chain of information is broken up due to the division of the functions, and its phases occur in different cells, then tissues without the process stopping. In these cells and tissues the result is a sort of differentiation. In the new organism produced through multiplication by the multicellular plants, during the period until the subsequent development of a new organism the phases of information branch as lateral chains of the informatory frame regarded as a cyclic process in the life of the individual.

In the above mentioned lateral chains, which through the occurrence of differentiation can be considered as the realization of the information, the quantity and quality of the information material characteristic of the individual have in several cases — e.g. in tissue cultures — been experimentally determined. The question arises, however, to what extent and in what time the various cell- and tissue types are able to activize their full supply of information. It may be put like that: to what extent is a certain type of cell or tissue able to regenerate, e.g. in the case of an injury, to recover its original form, or beyond its original form to produce e.g. a whole plant and with this activate hidden characteristics inherent in it? Thus we can say that information may enter in this case in various directions and various extents. Thus e.g. one of its forms is when the tissue zone preceding the injury develops from a callus tissue. In the other case the reactivization of the information is also partial; e.g. in tissue cultures from the callus there develops a different type of tissue. The reactivization process of information can be considered complete when e.g. from a cell there develops a whole plant, as in the case of the Beonia, or an organization form appearing as a new result: tobacco plant raised from a single pollen grain. And with this we have arrived at the question of the totipotence of cells which is related with the extent of the reactivization of the information stored in the cells.

The problem arises, further, that in the case of an injury, if a perfectly undifferentiated callus is formed, what is the determination mechanism that enters here like, as, in fact, in the case of a healthy plant it does not appear at all, we might say: it is not present, it is not contained in its information. It occurs only after the injury. And when an injury is not followed by callus formation, information does not enter. And here comes the experiment. Under the influence of a certain treatment callus formation begins. In this case an information phase was introduced from outside.

But let us see the tissue differentiation. It is the case of a multicellular plant organism As a result of fertilization, from a single cell two, then four, then at the early torpedo stage of the embryo a great many cells develop. The formation of the primordial organ has already started and the tissue differentiation has hardly — if at all — begun. Here the formation of the organ, as a higher organization unit, precedes the tissue differentiation. Thus, the realization mechanism too is at the cell, and not at the tissue level. Tissue differentiation is only a secondary process. But let us return to the basic question. In the course of the differentiation in the cells composing the young plant organism the information material is either divided into two, or some of its parts become blocked. Probably the latter is the actual process. If, on the other hand, we start from the two-celled stage and proceed to the four- then eight-celled stage, and so on, then this blocking occurs immediately before the differentiation, or in the eight- and four- or occasionally two-celled form, respectively, but may take place continuously during the process of cell multiplication, too.

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CAN THE TERM EPIGENESIS BE REPLACED BY THOSE OF GENETIC DETERMINATION AND REALIZATION MECHANISMS?

With reference to the paper dealing with the question of epigenesis I should like to emphasize that the precise definition of the fundamental conceptions used is considered to be one of the most important tasks of any field of science. Owing to the more than usual difficulties the biological sciences did not always solve this task as fortunately as e.g. the sciences of physics and chemistry. The author's initiation of this kind is, therefore, welcome. His solutions are, however, less satisfactory. While he is trying to clarify certain questions, new problematic ideas are thrown up in his exposition. I should like to pick out one of them.

The author obviously endeavours to separate the old historical *ideological fight* for preformist and epigenetic views from the study on the genetic process of epigenetic control. As regards the latter, however, putting aside the fairly appropriate term of "*epigenesis*" is — in my opinion — not the right solution; the *epigenetic process*, as a partial phenomenon of phenogenesis, should clearly be distinguished from the *epigenetic control*. Waddington (1960), Goldschmidt (1955), Mayr (1963), Elsasser (1962), and in a somewhat different respect Nanney (1958) and many others have been trying rather successfully to clear up the confusion of epigenetic *control*, and suggest the term "*epigenotype*" on this basis. They regard the epigenotype as *a norm, characteristic of the given organism, of the interactions between the internal and external environmental factors involved in the development of the genetic basis and phenotype.*

In our opinion, the epigenotype, and therefore, in an up-to-date sense epigenesis itself, as a process are of "epi" character for the very reason that they have a regulatory system in the background which — to some extent — is *above* the unrestricted manifestation of both the pure genetic determination and the environmental influences.

The epigenetic control — in our opinion — is no function of the extent, the information content transmitted by the parents manifests itself to, nor is it a function of the qualitative changes in the information content or of the informative effect of the environment, it is just the other way round, the epigenetic control is the coordinator of the manifestation of all these. This may be the explanation of the fact that in the ontogeny the realization of the genetic information can be relatively restricted and become mutually one-sided, while in the regeneration, or so called recapitulation phenomena it can fully or partially be extended again. And it explains too, that the phenotype is not a *restrained* but a *coordinated* and, to some extent, qualitatively modified realization of the genotype.

On the basis of the above we think that to accept the author's suggestion of putting the conception of epigenesis aside and speaking only of determination and realization mechanism would mean an undesirable simplification when analysing the role of the epigenetic system and the concept of epigenotype. The epigenetic control — like any kind of bioregulation — is an important *precondition* of the *appropriate realization*, and cannot be identified with the realization proper.

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WHY SHOULD THE PROBLEM OF THE GENETIC AND EPIGENETIC REGULATION OF THE DEVELOPMENT OF THE ORGANISM BE REVIEWED?

The paper of Dr. Kovács was written on the base of information accumulated during the time of the molecular biology central dogma elaboration. Now the central dogma of molecular biology is revised in connection with the reversed transcriptases discovery. That is why the problem of the genetic and epigenetic regulation of the development of the organism must be reviewed on the base of new facts and hypotheses in the field of investigation of RNA-regulation of DNA-synthesis.

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LECTIONES

CROSS-BREEDING WITH JERSEY IN ORDER TO IMPROVE THE NATIONAL BREEDS*

Having been honoured to talk on the topic of using the Jersey breed in improving the national breeds I shall try to do this from a virtually practical aspect mainly by using our own experimental data and at the same time endeavouring to avoid a documentation on this subject. I must, however, point to the fact that a very vast number of scientists have published papers on this topic especially in the last two decades. Referring only to some of the most outstanding ones the work of M. H. Fohrman, C. Wriedt, M. M. Lebedew, G. Schönmuth, etc. (see also annected references) should be mentioned. The publications — practically without exception — demonstrate a positive effect in nearly all cross-breeding experiments with Jerseys. From these data it may well be stated that the Jersey breeders have developed a breed, which in a number of characteristics ranks first, compared to all the modern breeds of the World. Their efforts led to a vast gen reserve, which I am sure will be of great use in the decades to come for rendering different cattle populations more efficient. The introduction of these gens into other breeds can also economize much time in the course of selection work. In this context may I draw the attention to the most important characteristics influencing milk production and represented in the Danish Jersey and one other breed which is considered today as one of the most popular milk producers: the Canadian Holstein. Table 1 shows that while the Canadian Holstein ranks first in milk quantity, the Jersey takes the lead in butterfat-, milk protein content, milk (4 per cent FCM) per 100 kg live weight which latter seems the most reliable indication of efficiency in overall milk production (Table 1). It would take many decades to develop these qualities to such a perfection in any breed. For this reason I think the statement of Lerner and Donald according to which "the Jersey and the Holstein Friesian breeds might well include all the gens which are needed in the future cattle breeding" is likely to be very near the truth.

Numerous experiments for improving milk production, the butterfat- and milk protein content of the milk, the udder conformation in certain breeds all show a very positive effect of the Jersey breed. The object of these experiments and breeding programs is generally to attain a certain Jersey gen-ratio, such as 12.5 per cent, 25 per cent, or 50 per cent. Such large cattle populations have been produced for example on our advice in the G. D. R., but also in a number of other countries. In our Hungarian breeding programs we produced populations with 75 per cent Hungarian Fleckvieh and 25 per cent Danish Jersey gen ratio, by keeping up the breed characteristics of the Fleckvieh. This population is created by using Jersey bulls with a colour-inheritance allowing to be dominated by the red and white of the Fleckvieh. See Fig. 1 representing the Danish Jersey bull Danaknaegt and his son from a Hungarian Fleckvieh cow (Figs 1, 2). This type of bull of the F_1 generation is mated to the Fleckvieh cows and this population

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* Report delivered at the World Conference of the Jersey Breeders on 3rd July, 1972 in Aarhus (Denmark).

Table 1

Average production of milk-recorded cow populations (1969—1970)

	Jersey	Canadian Holstein-Friesian
Milk kg	3707	*5690**
Butterfat kg	225	212
Butterfat %	*6.06**	3.73
Milk protein kg	159	188
Milk protein %	*4.30**	3.3
Fcm kg	4852	5460
Live weight kg	420	650
FCM/100 kg live weight	*1155***	840
Age at first calving, months	*24**	27

* This shows the great possibilities in combination
** The most evident factor of nutritional costs of milk production

Fig. 1. DANAKNAEGT imported from Denmark to Hungary has produced a great number of sons of Fleckvieh character and is considered the main founder of the "Hungarian milking Fleckvieh" breed with 25 per cent Jersey, 75 per cent Fleckvieh gen-ratio

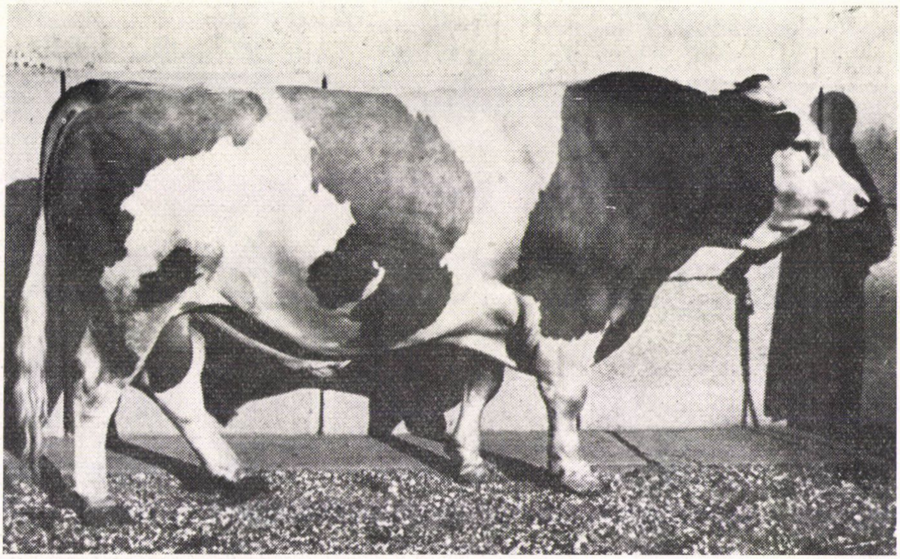

Fig. 2. Hungarian Fleckvieh × Jersey cross-bred F_1 bull used for recrossing with Fleckvieh

having 25 per cent Jersey gen ratio is then closed (Figs 3, 4). This population produces not only more milk, but also has 0.5 per cent more butterfat, 0.3 per cent more milk protein, a much superior udder conformation and milkability, the characteristics of easy calving and connected with this a better conception rate, as well as a 2—3 months earlier maturity than in the case of the Hungarian Fleckvieh. Concerning the beef producing qualities the hind quarters are somewhat less muscled, the deposition of fatty tissues starts earlier and food-conversion is reduced by 4 per cent. Nevertheless the overall beef producing capacity of this population is about 6 per cent higher than that of the pure-bred Fleckvieh, because the greater number of calves born and the earlier maturity of the stock more than compensates the mentioned negative effects.

Another population of cattle we produced in Hungary has 50 per cent of Danish Jersey gen-ratio. This stock has a superiority of 1 per cent butterfat, 0.5 per cent milk protein, an additional superiority in udder conformation and milkability, an earlier maturity by 4—6 months compared to the Hungarian Fleckvieh and a superior milk production (Fig. 5). The beef production of the individual animals is inferior to that of the Fleckvieh. The overall beef producing capacity of the whole population, however, ranks about the same as the Fleckvieh (101 per cent) due to the compensating effect of easier calving, earlier maturity. If utility cross-breeding is practised with 30 per cent of the female stock by using Hereford or Charolais breeds the beef producing potential of this population can further be increased. These cattle populations with Danish Jersey blood represent about 34.000 in Hungary today and will probably increase considerably.

Another population is now under construction which consists of 25 per cent Danish Jersey and 75 per cent USA-Canadian Holstein. These cows of the experiment are actually producing an overall of 5000 kg milk in the second lactation with 4.3—4.4 per cent butterfat and 3.4—3.6 per cent milk protein (Figs 6, 7). This population is especially useful in building up dairy herds around the milk consuming centres.

In connection with the production of the Jersey breed the relative production (4 per cent FCM per live weight unit) which has a considerable bearing on cross-breeding must be

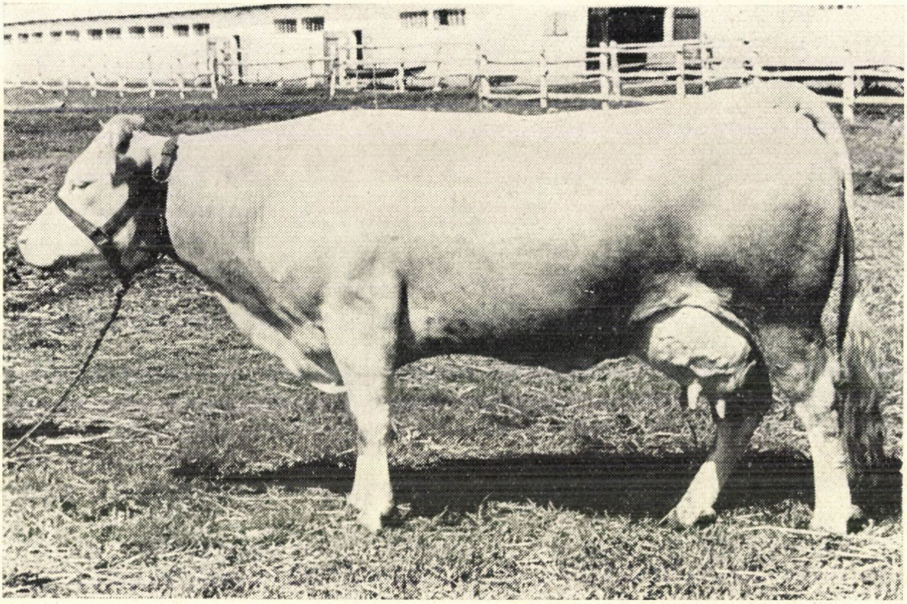

Fig. 3. Hungarian milking Fleckvieh cow CSACSKA. Prod: av. of 4. lact. in 300 milking days: 8050 kg milk, 342.3 kg fat (4.25 per cent); max. prod.: 9380 kg milk, 428.8 kg fat (4.57 per cent)

Table 2

Ratio per breed of cows attaining 3000 kg butterfat production in Denmark (1969/70)

Breed	Among the milk recorded cows belong to the breed, %	Among all cows producing more than 3000 kg butterfat belong to the breed, %	Live weight of the cows, kg	Average milk yield standardized 4% butterfat (FCM) per 100 kg live weight of the breed
Danish red	38.8	*12.6* (n=30) (Av. 3347 Bf, kg)	580	860 (100%)
Danish Friesian	42.0	*19.7* (n=47) (Av. 3249 Bf, kg)	610	803 (93%)
Danish Jersey	18.8	*67.6* (n=161) (Av. 3453 Bf, kg)	420	1155 (134%)

dealt with. In pure breeding this may be demonstrated in Table 2 which I worked out for a European meat and milk study of FAO and which shows the data of the cows having produced more than 3000 kg butterfat by the three leading breeds in Denmark (Table 2). It can be seen that comparing the three breeds (Red Danish, Danish Friesians, and Danish Jersey), although, the Danish Jersey only represents 18.8 per cent of the total stock, 67.6 per cent of the cows having

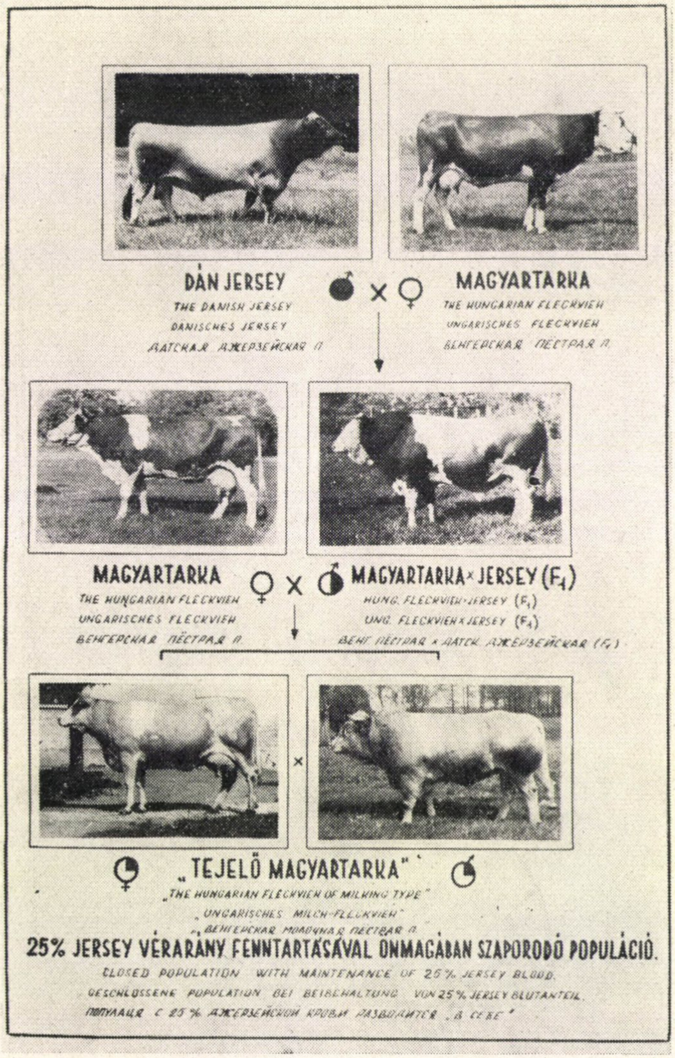

Fig. 4. Scheme of the production of the "Hungarian milking Fleckvieh" with 25 per cent Jersey gen ratio

attained 3000 kg butterfat production belong to this breed. The superiority is even more striking if we express this production in milk yield standardized to 4 per cent FCM per 100 kg live weight. This characteristic is the most important if we look for efficiency in milk production. If we take the Red Danish for 100 per cent in this respect (860 kg of 4 per cent FCM) than the Danish Friesian produced 93 per cent (803 kg FCM) and the Jersey 134 per cent (1155 kg FCM). I think that this characteristic of the Jersey breed is not taken into consideration according to its due merit. The high relative production of the Jersey works out most strikingly in crossbreeding too. Yet this is an item which is also not taken into account even by renowned scientif-

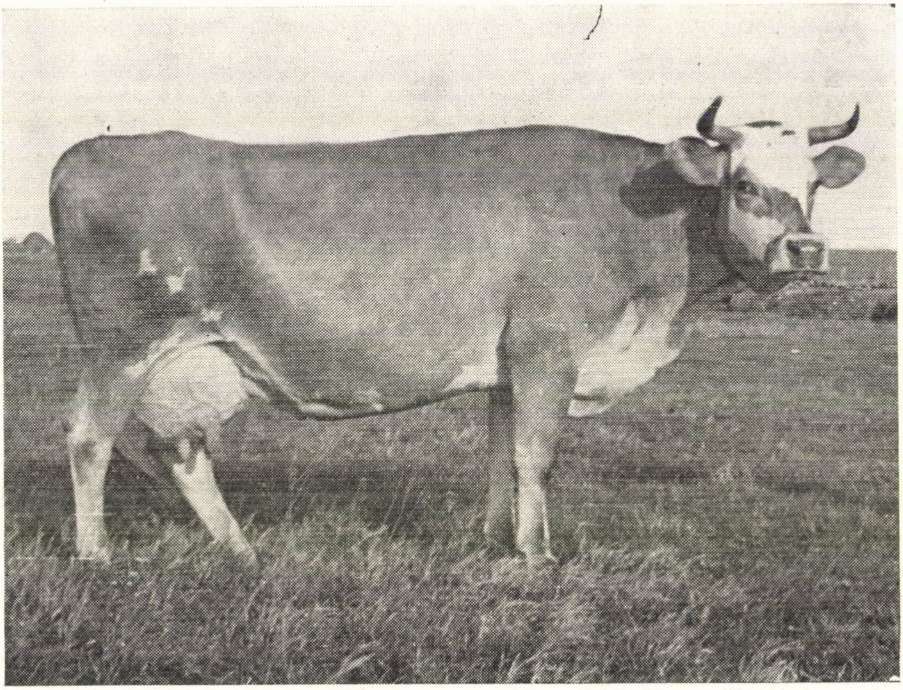

Fig. 5. Hungarian brown cow of 50 per cent Jersey gen ratio. Max. prod. in 300 milking days: 6847 kg milk, 365.2 kg fat (5.33 per cent)

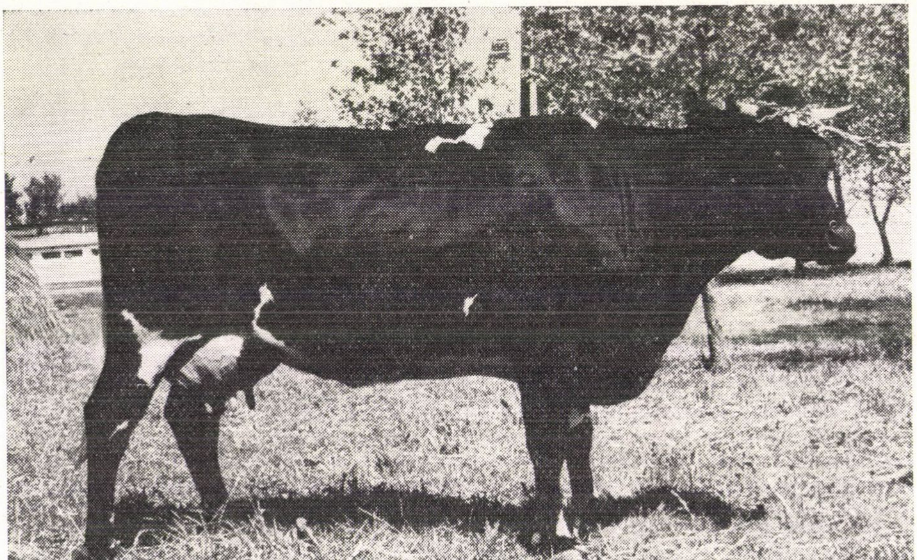

Fig. 6. Cow of the population 75 per cent Canadian Holstein and 25 per cent Danish Jersey

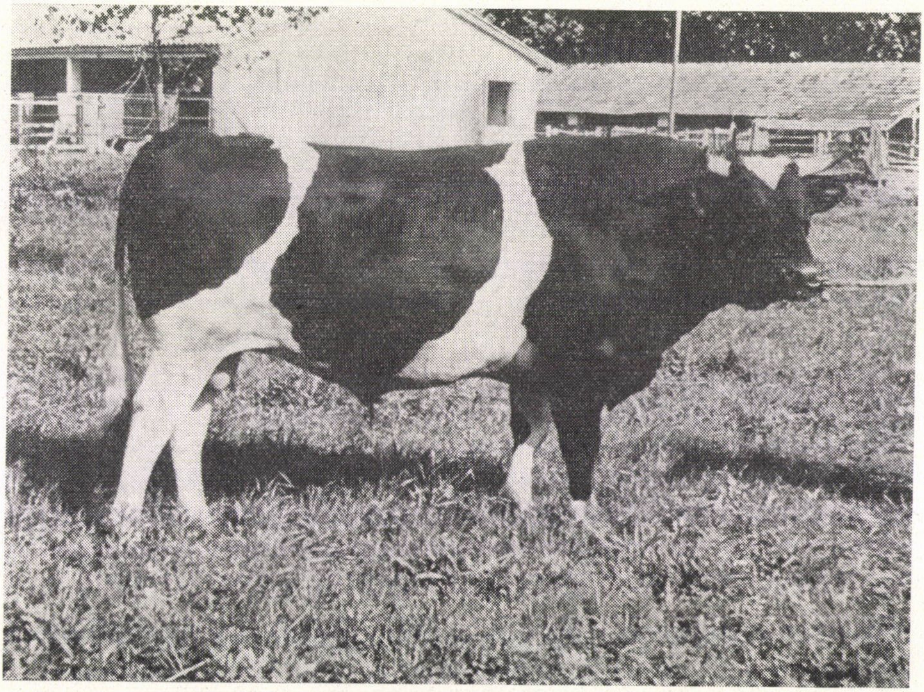

Fig. 7. Bull of the population 75 per cent Canadian Holstein and 25 per cent Danish Jersey

ic workers who generally only deal with the absolute figures of milk quantity, milk concentration, milkability, etc. For this reason it may be of special interest to visualize the big difference of the absolute production and the relative production (related to 100 kg live weight) in the Hungarian experiments (Table 3). The great superiority of the cross-bred stock over the pure-bred Hungarian Fleckvieh is clearly demonstrated in three lactations. This superiority expressed as a percentage is in the I lact. + 39 per cent, in the II lact. + 33 per cent and in the III lact. + 39 per cent. If we look at these figures in connection with relative production which means real efficiency, than we find in the I lact. + 71 per cent, in the II lact. + 62 per cent and in the III lact. + 75 per cent. It may well be stated that the superiority of the cross-breds is nearly doubled if you take into consideration the relative production. It follows that the Jersey breed can not only transmit its considerable absolute producing superiority, but also its outstanding producing capacity per live weight unit. This means that the Jersey breed transmits very important physiological and endocrinological characteristics, which realized in a larger cross-bred organism often also demonstrating hybrid-vigor leads to these outstanding results, which can be registered in all cross-breeding experiments although generally not full justice is paid to this very important item. Our large experiments which have been repeated several times strikingly reflect this superiority in food conversion as regards milk production. This means that for example the stock of 50 per cent Jersey gen ratio produces butterfat by 30 per cent, milk protein by 20 per cent less starch equivalent than the Hungarian Fleckvieh.

A very remarkable and at first hearing puzzling fact is furthermore that the Jersey may not only play an important role in improving the milk producing ability of different breeds, but also introduce some characteristics into certain populations which improve the overall beef

Table 3

The difference between the absolute and relative production

Breed	Live weight, kg	Production expressed on 4% FCM		Production of 4% FCM per 100 kg live weight	
		kg	%	kg	%
Hung. Fleckvieh					
I. Lact.	598	2481	100	415	100
II. Lact.	647	3394	100	526	100
III. Lact.	676	3479	100	515	100
Hung. Fleckvieh× ×Dan. Jersey F_1					
I. Lact.	487	3450	139	708	171
II. Lact.	530	4520	133	853	162
III. Lact.	536	4839	139	903	175

Table 4

*Some breeds of outstanding gen-combinations in milk and beef production**

	Characteristics important especially in milk production				Characteristics important especially in beef production			
Breed	Milk quantity	Fat %	Protein %	Udder conf. + milk-ability	Daily gain	Early matur-ity	Calf prod.** Cap Food/ unit	Slaugh-ter value***
European Friesian	+++	++	++	++	+++	+++	+++	+++
US-Canadian Holstein	++++	+	++	+++	++++	+++	+++	++
Jersey	+++	++++	++++	++++	+	++++	++++	+
Fleckvieh	++	++	++	+	++++	++	++	++++
Hereford	—	—	—	—	+++	+++	+++	+++
Charolais	—	—	—	—	++++	+	+	++++
Angus	—	—	—	—	++	++++	++++	+++

* Signs: ++++ excellent; +++ good; ++ medium; + poor;
** A) Low cost of maintenance per cow; B) Easy calving
*** Evaluation partly on subjective grounds

producing efficiency. To demonstrate this fact it may be of interest to show a table (4) worked out for a FAO study on milk and beef production in Europe. This Table 4 shows some well known breeds of outstanding characteristics (Table 4). Out of the listed 8 qualities the first 4 serve mainly for the efficiency of milk production, the second 4 on the other hand are especially

Fig. 8. Heifers descending of utility cross-breeding the "Hungarian brown" with Hereford. The heifers have a 25 per cent Jersey, 50 per cent Hereford gen ratio and serve as suckler cows

important in beef production. The different European breeds show a manifold combination of the enumerated traits. This is especially true for the dual purpose breeds. It may be clearly seen from the evaluation of even the most outstanding breeds, that there is no ideal dual purpose breed, but also no beef breed, which is up to the requirements in all respects. If the capacity of growth rate is satisfactory, the efficiency for calf production will fall behind. In milk production the situation seems less contradictory. It may also be seen that the Jersey breed not only ranks between the best in milk production, but also has two characteristics, of the 4 important ones which are highly estimated in the field of overall beef production. This is the reason why in recent years the Jersey breed also seems to be an attractive partner in producing a population of suckler cows. The small body size, early sexual maturity, ability to give easy-birth even if inseminated with the semen of large sized breeds (Charolais, Simmental, etc.), satisfactory milk yield, high milk concentration which, it seems, is less influenced by unfavourable environmental conditions, are all important qualities for a suckler cow stock. The efficiency of the suckler cow depends on the quantity of nutrients consumed per weaned calf per year. In more practical terms the suckler cow which weans the biggest calf weight per live weight unit will be the most efficient. Such a cow should be of low maintenance cost, early maturity (calving at 22—24 months of age), a satisfactory milk producer and an easy calver. All these traits are characteristic for the Jersey. This is the reason why the Jersey breed will probably also become important in producing a suckler cow stock. Some data on this aspect were mentioned, when I talked about our Jersey cross-bred populations by giving their beef producing potential. In our further experiments the heifers descending from a utility cross-breeding of the 50 per cent Danish Jersey population with Herefords serve as suckler cows (Fig. 8, 9). These can be produced and used very economically:

1. There is no necessity for fattening heifers which is generally an uneconomic procedure;
2. The heifer stock for replacement need not be specially produced by pure-breedings

Fig. 9. Suckler cow calved at 22 months (25 per cent Jersey, 50 per cent Hereford gen ratio

3. and the heifers can be cross-bred throughout their utilization with the most convenient beef breed e.g. for the first calving with the Hereford, or Angus and later with Charolais or Limousin, etc.

Our first experimental results are very satisfactory in this respect. The heifer produced in this way (25 per cent Jersey, 50 per cent Hereford, and 25 per cent Fleckvieh) sired at first by a Hereford has a capacity of calving down without any casualties at the age from 21—24 months (average calving age was 21.9 months). The weight of the calves according to the first calving age of the mother was:

At 19 months of age	26.7 kg	(n = 9)
At 20 months of age	29.0 kg	(n = 2)
At 21 months of age	28.5 kg	(n = 2)
At 23 months of age	20.0 kg	(n = 3)
At 24 months of age	25.0 kg	(n = 1)
At 27 months of age	25.0 kg	(n = 1)

According to the opinion of the farmers it would not be difficult to let these heifers calve down at the ages of around 20 months without having to fear calving difficulties or a too much reduced milk production.

As far as milk production is concerned two items are of interest: 1) The quantity of milk of which only estimations are available but they indicate that these cows produce a quantity of 2000—2500 kg. 2) The concentration of the milk. Although the cows have not been milk-recorded systematically the few tests during the lactations indicate that such heifers have a milk concentration of 4.3—4.4 per cent butterfat and 3.6—3.8 per cent milk protein. This seems to be very important — also in such areas where the rainfall may sometimes be scarce, as the concentrated milk producing stock, as already mentioned, seems less affected by unfavourable weather conditions. This system of combinative breeding is shown in Table 5 and may be useful in making beef production more or less independent from milk production on a very economic basis (Table 5). This system of breeding and the suckler cow keeping may be economic if the milk-beef price ratio is getting under European conditions near 1 : 7 — 1 : 8.

It may be stated from the shortly outlined experiments as well as the analyses of different characteristics in cattle breeding that the Jersey breed has a very important part to play in the formation of the cattle of the future. This is probably true not only in milk production but also as far as beef production is concerned. In this context I must, however, point to the fact, that pure breeding and cross-breeding are no competitors. There is only a timely succession in these breeding systems. There is no effective and systematic cross-breeding without well founded pure-breeding. The Jersey breeders of the World have therefore not only the great task of further developing their excellent breed, but also to render it more and more adequate to be used for improving the national breeds of the different countries of the World.

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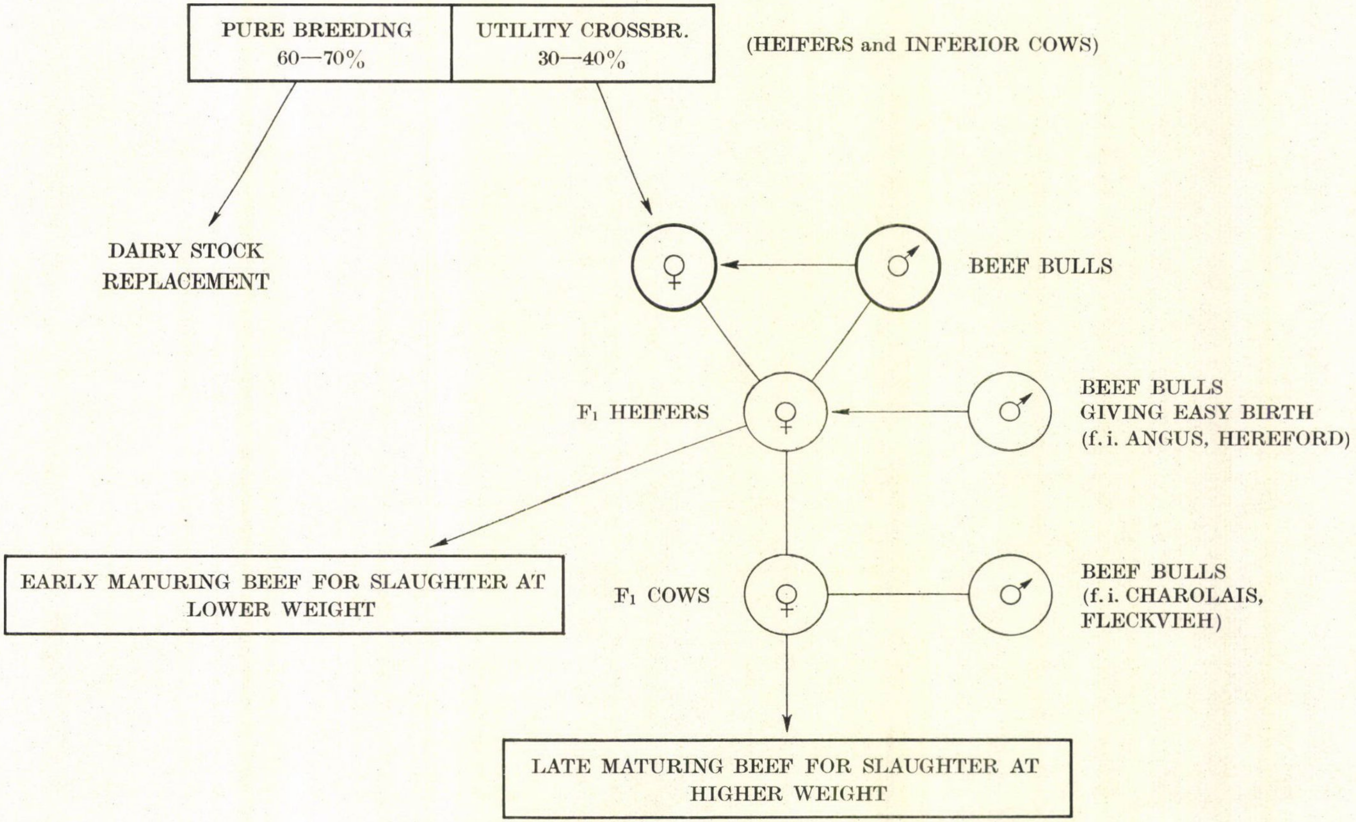
Table 5


PURE BREEDING
60—70%
UTILITY CROSSBR.
30—40%
(HEIFERS and INFERIOR COWS)
DAIRY STOCK REPLACEMENT
♀
♂
BEEF BULLS
F₁ HEIFERS
♀
♂
BEEF BULLS GIVING EASY BIRTH (f. i. ANGUS, HEREFORD)
EARLY MATURING BEEF FOR SLAUGHTER AT LOWER WEIGHT
F₁ COWS
♀
♂
BEEF BULLS (f. i. CHAROLAIS, FLECKVIEH)
LATE MATURING BEEF FOR SLAUGHTER AT HIGHER WEIGHT

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PHYTOTRON AT MARTONVÁSÁR FOR ELUCIDATING RELATIONSHIP BETWEEN METABOLISM AND HEREDITY*

The present paper is to discuss briefly the initial steps of our autumnization genetic research which led to the idea of the Martonvásár Phytotron, the air-conditioned chambers of our own making established since the early sixties and the design of the Martonvásár Phytotron under construction as well as its research orientation.

1. At a Jubilee Scientific Conference held at Martonvásár in December 1959 upon celebrating the 10th anniversary of the foundation of the Agricultural Research Institute of the Hungarian Academy of Sciences, a report summarized the results of the first four years of our autumnization genetic experiments (RAJKI 1961). Therein it was stated that in an initial stock, proved by conventional genetic analysis to be pure spring wheat, under environmental conditions produced by appropriate combinations of autumn sowing times genetically pure winter wheat plants developed.

On analysing the causes of autumnization we came to conclude during the discussion taking place at the above-mentioned scientific conference that both for establishing the proper content of environmental conditions determined by "appropriate" autumn sowings and to reproduce exactly these experimental circumstances, phytotron investigations are necessary. This is why we decided upon the establishment of the Martonvásár Phytotron.

2. Simultaneously, in order to meet research requirements plant growth chambers were made during the sixties in three steps through the co-operation of scientists and workshop technicians of our Institute.

Thus, under our Institute's central water-basin in 1960 a roughly conditioned room was established in which temperature ranged between 5 and 12 °C in winter and between 15 and 22 °C in summer. Day-length was regulated by a switching clock and light spectrum by cellophane filters.

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* Lecture delivered on May 27, 1972 at the Duke University, Durham, on June 22, 1972 in the All-Union Genetics and Breeding Institute, Odessa, on June 24, 1972 in the Research Institute for Wheat Breeding and Seed Production, Mironovka and on June 27 and 28, 1972 in the All-Union Institute for Plant Production, Leningrad and Pushkin.

In 1964 we constructed a more advanced growth chamber, wherein conditioning of temperature has been possible, during the first years, between 0 and 25 °C, but since 1969, by means of a built-in defrosting system, down to —5 °C, too. For lighting mercury lamps while for regulating light spectrum filters and high-voltage neon tubes have been used.

Two more air-conditioned chambers were constructed in 1967. One of the chambers was installed for vernalization in which temperature ranges between 0 and 2 °C and the necessary lighting is given with high-voltage neon tubes. The other is a plant growth chamber, in which air temperature can be regulated between 5 and 25 °C and day-length as desired. The distance of plants from a light canopy consisting of fluorescent tubes is adjustable by raising or lowering the movable benches. In the plant growth chambers in the three steps (1960, 1964, 1967) light intensity at a distance of 50 cm from the light canopy, is 8 000 to 10 000 lux.

3. In the growth chambers established in 1960 and 1964 basic research on vernalization biochemistry and autumnization genetics and in the air-conditioned chambers established in 1967 mainly basic research on aneuploid and autumnization genetics have been conducted. These investigations have significantly contributed to elucidate the biochemistry and genetics of the autumnization process. It was proved (RAJKI 1967, RAJKI—RAJKI 1969) that the various environmental factors are able to exert their adequate effect on the nature of plant metabolism. This effect as an "information" can pass through the metabolic levels and the result of their interactions may become fixed in the reproductive cells as a new or modified DNA — a new inheritable property of the organism. In contrast with one of the two general principles of the molecular gene concept, that is Crick's central dogma (CRICK 1958), which, besides certain recent writings of the earlier "Western Debaters" (COMMONER 1968, CHARGAFF 1971), has been debated for the past year and a half by microbial geneticists too (TEMIN—MIZUTANI 1970, etc.), we have repeatedly postulated (RAJKI—DÉVAY—RAJKI 1970) the bilateral transfer of genetic information which was rendered probable for the first time six years ago (RAJKI 1966) on the basis of our autumnization research. Moreover, in our recent treatise (RAJKI—DÉVAY—RAJKI 1972) an attempt was made to further develop the metabolism-biochemical concept of heredity and to make its necessary demarcation both from the molecular gene concept and the Michurinian genetic concept.

4. During the preparation of the Martonvásár Phytotron, in the sixties several West European and North American phytotrons were studied and on the basis of experiences gained both in the growth chambers of our own making and in the foreign phytotrons the Martonvásár Phytotron concept was formed. The expression of phytotron is used for a set of plant growth chambers[1] and/or cabinets[2] designed for a definite research program which may be completed with air-conditioned green-houses and artificially illuminable fields, too.

In the Martonvásár Phytotron under construction in the making of our Experimental Farm's building team, the air-conditioned green-houses are in part substituted by plant growth benches on which — uncontrollable sunlight being excluded from the rooms containing them — plants are to be grown under artificial lighting (nominal light intensity — 25 000 lux) which can be programmed in three intensity steps. The entire growing area of the fourteen plant growth benches is 60.2 m^2. It may be mentioned here that for the last fifteen years already we have been in the possession of an artificially illuminable field.

Twelve plant growth chambers and sixteen cabinets are the heart of the Martonvásár Phytotron. Their entire growing area is 62 m^2: 39.6 m^2 for the chambers and 22.4 m^2 for the cabinets. The chambers are of "spring–autumn" type with a temperature controllable between —5 and 40 °C. The cabinets are of "summer" type with a temperature controllable between 5

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[1] The growth chamber is bigger than the cabinet and the operator can enter it.

[2] The growth cabinet is smaller than the chamber and the operator reaches into it to handle plants.

and 40 °C. The nominal light intensity both for the chambers and cabinets is 50 000 lux, but for four cabinets — up to 100 000 lux which is equal to the maximum summer light intensity. In these units too light intensity can be programmed in three steps, but individual manual switches will give lighting increments of 1/15th.

To the plant growth units two frost-resistance testing or "winter" chambers are added with a useful area of 14.2 m^2. In these chambers the minimum temperature to be programmed is —20 °C.

Except the two "winter" chambers in all the 42 plant growth units humidity conditions can also be programmed.

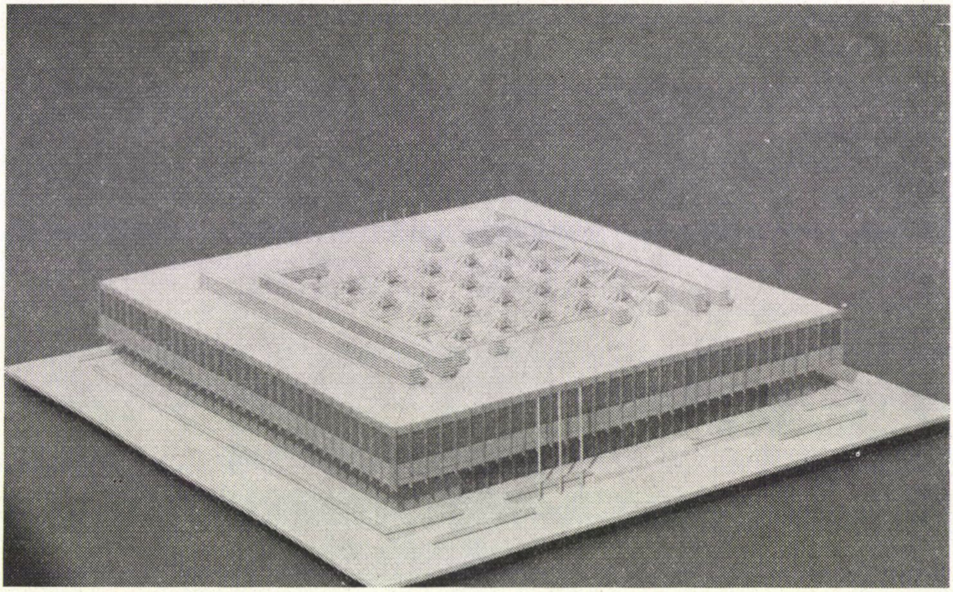

Fig. 1. The maquette of the Martonvásár Phytotron

In our air-conditioned units the maximum deviation from the programmed value cannot be more than ±0.5 °C for temperature, ±5% for light intensity and ±3% for humidity at any point in one plane, at any time. All the units are self-contained with built-in air-conditioning systems which automatically correct deviations from the programmed temperature and humidity. Deviations from the programmed light intensity can be corrected by raising or lowering the lamp fixture. The plant growth/testing chambers, cabinets and benches were delivered by Conviron, Winnipeg, Canada.

The Martonvásár Phytotron is a two-storey structure (Fig. 1.) 50 × 50 m, in which a central hall 30 × 30 m is situated in the centre of its second storey for housing the air-conditioned units. Air, water and electricity supply of the 44 air-conditioned units of the central hall is managed from rooms situated under the central hall. On two stories around the central hall laboratories for preparing experimental plant material (seed testing, sterilizing, vernalizing, storing, germinating, etc.) and for processing and further examining plants originating from the air-conditioned units (cytological, histological, sterile conditions, chemical preparatory, etc.) as well as rooms for the staff and other facilities (workshops, shower-baths, etc.) are situated.

The ceremonial opening of the Martonvásár Phytotron will take place — after due trial operation which has been going on since early April — on November 3, 1972.

5. As to the research orientation of the Martonvásár Phytotron we started from our original phytotron concept. Accordingly, the Martonvásár Phytotron is aimed at the exact scientific elucidation of the relationship existing between metabolism and heredity, and the objective answering of this cardinal question in biology, genetics and phylogenetics. The latter will endeavour, at the same time, to establish scientifically the elaboration of exact methods for controlling certain major agronomic characters, first of all, winter and spring types as well as frost-resistance and winter hardiness, in favour of practical plant breeding.

As a basis for programming temperature, lighting and humidity in the phytotron, field and other experiments of the past one and a half decades, or rather their temperature, lighting and humidity tracings, will serve. The possibility for such solution can be well demonstrated by the autumnization at Martonvásár of certain Mexican spring wheats bred by Dr. Borlaug. The latter experiment started in the autumn of 1969 by using a method of autumnization which was elaborated at Martonvásár in field and growth chamber experiments as well as by investigations conducted in our cytological and physiological laboratories (RAJKI 1967, RAJKI—RAJKI 1969, RAJKI—DÉVAY—RAJKI 1970, 1972).

6. This autumnization experiment has been carried out in order to obtain winter hardy semi-dwarf basic material for wheat breeding and to control the reproducibility of the autumnization research results.

The Mexican spring wheats were selected, on the basis of the first year results of their performance test (RAJKI—PÁL 1972), from those cultivars which seemed to be the most perspective in spring sowing under local conditions. The cultivars under study are as follows: Super X, Penjamo 62, Siete Cerros 66, Nainari 60 and Azteca 67. Together with these Mexican spring wheats the standard Hungarian spring wheat cultivar Kompolti szálkás and the Russian Bezostaya 1 as a winter wheat control cultivar too have been sown.

The method employed for timing the sowings is illustrated in Table 1. Here the sowing time combinations of September 1971 are shown only, nevertheless the sowing time combinations of October 1971, November 1971 and spring 1972 also correspond to these.

Hundred plants were taken from each plot in the summer of 1970 and also in 1971 and the following year experiments were sown with the seed blend of their first spikes. This was modified in the summer of 1971 in that the first spikes mentioned were broken into four parts and so the seed blend of these hundred quarter-spikes were sown in September, October and November 1971 and at the beginning of April 1972.

In three rows, four meters long each, 320 seeds per row were sown in every sowing time treatment in 1969/70 and 1970/71. Owing to the great number of variants a single four meter row with 320 seeds per combination produced one plot in 1971/72.

From each treatment of the September 1971 sowings several plants were replanted into pots on November 22 and raised in a growth chamber at a temperature of 18—22 °C.

Along with the four series (September, October, November, spring) of the 1971/72 sowing time treatments, a corresponding fifth series too was raised in a growth chamber. This was sown on November 25 and plants were grown up at a temperature of 18—22 °C.

Growth habit was determined on the basis of the type of young shoots as well as of the heading of spring and growth chamber sowings.

In the growth habit of experimental plants there was no change whatsoever indicated both by the type of young shoots and heading in the first and second years of the autumnization experiments of the Mexican spring wheats. Due to the mild winters and the fair snow-cover there was no notable winter damage in the autumn sowings.

The first signs of change in the type of young shoots appeared in mid-November 1971 in several September sowing time treatments of certain cultivars. According to the results of

Table 1

Sowing time combinations of September 1971

Martonvásár, 1971/72

Sowing time combinations	69/70	70/71	71/72
Earliest autumn	S	S	S
Early autumn	S	O	S
	S	N	S
Mid-early autumn	O	S	S
	O	O	S
	O	N	S
Late autumn	N	S	S
	N	O	S
	N	N	S
Autumn combined with spring sowing	T	S	S
	T	O	S
	T	N	S
	S	T	S
	O	T	S
	N	T	S
	T	T	S

S = September O = October N = November T = Spring

examinations, autumnization took place in certain cultivars (Nainari 60, Kompolti szálkás), especially noticeably in the Mexican spring wheat Penjamo 62, in fact, in all the test plants of the earliest autumn sowing combination. The change in the growth habit completed for the third year in the earliest autumn sowing time treatment of Penjamo 62 was also indicated by the heading of plants replanted into the growth chamber on November 22 (Table 2). Concerning the change in the growth habit of Penjamo 62 the heading of plants in the earliest sowing time treatment of the fifth series sown on November 25 in the growth chamber, more exactly, the lack of heading in the earliest autumn sowing time treatment, is an even more convincing proof (Table 3). In some early autumn sowing time combinations of Penjamo 62 autumnization was observable in approximately one third of the test plants. At the same time practically no autumnization occurred in late autumn and none at all in those autumn sowing time treatments which were further combined with spring sowing.

Over-wintering corresponded to autumnization, i.e. in general autumnized plants survived only. Certain temperature and snow-cover values of the 1971/72 winter are shown in Table 4. The 1971/72 wintering was characterized both by the almost complete lack of a fair snow-cover and the extreme fluctuation of negative and positive temperatures, the latter even in the pre- and post-winter periods.

The morphology of the autumnized Penjamo 62 plants is similar to that of the initial spring Penjamo 62 plants.

Table 2

Heading of plants replanted from nursery into growth chamber
Martonvásár, 1972

Cultivar	Sowing time			Heading
	1969/70	1970/71	1971/72	
Penjamo 62	September	September	September +Phytotron*	Feb. 5
Penjamo 62	Spring	Spring	September +Phytotron*	Jan. 22
Bezostaya 1	September	September	September +Phytotron*	Feb. 13

* Plants replanted from nursery into growth chamber on November 22.

Table 3

Heading in growth chamber
Martonvásár, 1972

Cultivar	Sowing time			Heading
	1969/70	1970/71	1971/72	
Penjamo 62	September	September	Phytotron	—
Penjamo 62	Spring	Spring	Phytotron	+
Bezostaya 1	September	September	Phytotron	—

Table 4

*Wintering conditions**
Martonvásár, 1971/72

	October	November	December	January	February	March
Number of days with a radiation minimum temperature below —10°C	2	3	1	11	4	2
Least value of radiation minimum, °C	—13.5	—13.6	—10.2	—18.2	—12.5	—13.2
Maximum air-temperature, °C	23.0	18.3	14.5	5.0	12.0	20.7
Deviation of the monthly mean values of air-temperature from the many year averages, °C	—0.7	0.4	3.2	—0.5	2.9	2.5
Number of days with a fair snow-cover	0	5	0	4	0	0
Deviation of the number of days with snow-cover from the many year averages	0	4.5	—9.7	—13.1	—10.5	—2.9

* Data of the Martonvásár Agro-Meteorological Observatory of the National Meteorological Institute (Pletser J.).

7. Autumnization observed in the earliest and early autumn sowing time combinations of the Mexican spring wheat Penjamo 62 was in full agreement with earlier conclusions reached in the course of the fundamental research in autumnization at Martonvásár.

With the genetic interpretation of the mentioned facts of autumnization it should be borne in mind, that the earliest and early autumn as well as the mid- and late autumn sowing time treatments have so far "experienced" the same three winters. This — taking into account also the estimated rate of autumnization as an adequate genetic conversion — casts *a priori* doubts on the validity of interpretations founded on original heterogeneity and/or mutation. Certainly no comment is required to interpret these facts from either breeding methodology or practical wheat breeding points of view.

In the Martonvásár Phytotron under construction there will most certainly be probable both to reproduce the changes in certain environmental conditions and to carry on the exact testing of their individual effects to which autumn-sown spring wheats are exposed. In the course of the autumn vegetation period they are as follows: gradually decreasing temperature and light intensity, shortening day-length, a spectrum becoming rich in red, i.e. those which are otherwise diametrically opposed to the corresponding tendencies prevailing in spring. From these and similar investigations the elaboration of an autumnization "recipe book" for cultivars to be converted as well as for planned winter type, and winter- and frost-resistance is to be expected.

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CHRONICA

LÁSZLÓ VARGHA

1903—1971

On July 1, 1971, László Vargha, Member of the Hungarian Academy of Sciences, formerly professor of organic chemistry at the Hungarian "Bólyai" University of Kolozsvár, director of the Pharmaceutical Research Institute, passed away. His death has been a great loss for the Hungarian scientific research in organic and pharmaceutical chemistry.

László Vargha was born in 1903 at Berhida, where his father was a Calvinist pastor. His mother died when he was a child, therefore, he did not go to secondary school in the nearest town but studied in the famous Presbyterian Gymnasium at Pápa; here he found a second home in the house of a relative. After graduating from secondary school with an "excellent" grade his intention was to attend the Faculty of Chemistry at the József Technical University of Budapest as his interest in chemistry was aroused already by his excellent high school teacher who assigned him to the preparation of class experiments. The Technical University, however, referring to the restriction imposed on the number of students, rejected his application, so he was enrolled at the Faculty of Philosophy, P. Pázmány University, where he mainly pursued chemical studies. Although at the beginning he had been disappointed by not being admitted to the Technical University, his experience at the Pázmány University soon convinced him that there he could carry on studies corresponding with his inclinations, and later he was even convinced that the rigid curriculum of the Technical University would have hardly given him opportunity to concentrate on scientific disciplines he was most interested in. It was a fortunate turn of events that at the University, where no department of organic chemistry existed at the time, Jenő Pacsu, assistant and private docent (later an eminent professor at Princeton University) began work in organic chemistry in an almost autodidactic way. As a senior student, Vargha obtained a position in Pacsu's laboratory and, after completing his fundamental studies in theoretical and practical organic chemistry, prepared his Ph. D. thesis under the direction of Pacsu on acyl migration during the partial saponification of diacylprotokatechualdehyde[1]*. In 1926 he graduated as Ph. D. in chemistry as his major subject and in physics and geology as minor subjects, with First Class Honours ('summa cum laude'). Upon the recommendation of Pacsu he was employed as private assistant with a modest salary in the laboratory of Prof. G. Zemplén who, a year later, helped him to obtain a travel grant with state scholarship. In possession of this, as a member of the Collegium Hungaricum, he was engaged in carbohydrate research for two years at Berlin University as a coworker of Prof. H. Ohle. The time spent in Berlin proved to be very fruitful [2—5], and the success of his work made the chemistry of carbohydrates extremely attractive for Vargha. The intimate and friendly relationship with his professor and the vivid scientific life in E. Fischer's former institute and the Berlin University in general undoubtedly contributed to this.

With the termination of his Hungarian state scholarship, Vargha was doubtful of obtaining employment at home, therefore, on the proposal of Prof. Ohle, he accepted a post of private assistant offered to him by Prof. A. Schönberg. This position brought him to the Institute of Organic Chemistry, Technical University of Berlin-Charlottenburg, where, however, instead of carbohydrates he performed studies on organic sulfides. He soon became familiar with this new subject as witnessed by the papers published in the course of the two years [6—11].

Returning after four years of successful work abroad he shared the lot of the young intellectuals of those days: he had to live for years on occasional Hungarian scholarships of very modest size. He worked for a year at the Institute of Medical Chemistry, Ferenc József University, Szeged, as an associate of Prof. A. Szent-Györgyi; then for a year at the Tihany Biological Research Institute as a substitute for Sándor Müller who was staying at that time in England on a Rockefeller fellowship. This was followed by two years at the Szeged University, this time at the Institute of Organic and Pharmaceutical Chemistry headed by Prof. Tibor Széki; finally, he spent one year at the Pázmány University, at the Biological Institute directed by Prof. Aladár Beznák. While these underpaid occasional employments only provided for bare subsistence, they still rendered the unfolding of Vargha's talent possible. This is proved by the papers published at that time [12—24], which made his name known also in professional circles abroad. These papers were mainly concerned with the results of his independent research on carbohydrates. This activity was acknowledged by the Faculty of Natural Sciences of the Ferenc József University in 1935 when Vargha was qualified as a private docent in the subject of the "Chemistry of Carbohydrates".

In spite of this well-deserved moral recognition he still could not find a regular employment which would have permanently provided a better living. It was in 1936 that the first opportunity was offered to him when he undertook the organization and management of the research laboratory of synthetic organic chemistry in the Gedeon Richter Chemical Factory, temporarily giving up his favourite field of basic research in organic chemistry. However, the years spent at this post proved very useful since his attention was called to the chemistry of pharmaceuticals and his views about the close connection between science and industry were

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* The numbers in brackets refer to the list of Vargha's publications.

confirmed. In his opinion, without basic research in organic chemistry the Hungarian pharmaceutical industry had no real prospects of development.

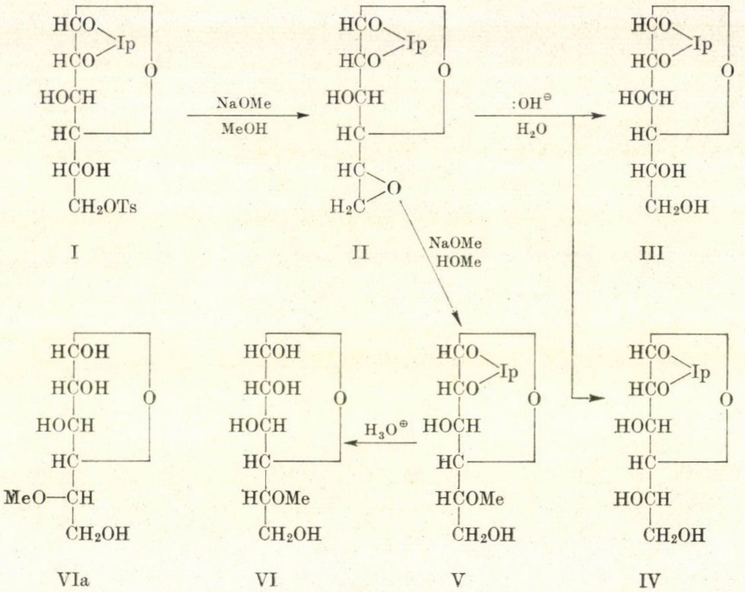
The possibility of a wider range of basic research in organic chemistry opened up again at the end of 1940. He was appointed professor of the Ferenc József University resettled from Szeged to Kolozsvár, and director of the Institute of Organic Chemistry. Here the modernization of the old institute and the organization of education and research imposed much work on him, and he could enjoy the results of his work only for a short time, because in 1945 he had to resign in favour of Prof. Tanasescu. In consideration of his services, he was appointed by the Roumanian educational authorities professor of Hungarian citizenship at the newly established Hungarian Bólyai University in Kolozsvár. His task was to develop the new university institute of organic chemistry in a section of a building constructed to house a secondary school; besides, he took active part in organizing the whole of the Bólyai University. This was a difficult task in the years immediately following the war. But hardly was this work completed, in 1950 the Roumanian government did not renew the contract previously extended on a yearly basis, thus László Vargha, after 10 years of activity as a professor at Kolozsvár, faced again the worries of finding employment elsewhere.

After returning to Budapest in October 1950 he was appointed head of section, then in 1957 director of the newly established Hungarian Pharmaceutical Research Institute, and remained at this post up to the end of his life, that is for fifteen years. At the time of his appointment to head of section, the institute operated temporarily in an apartment house and it was during Vargha's activity as a director that the new headquarters of the institute with its perfectly equipped laboratories were built. Heading this project, he again gave evidence of his abilities as an organizer; he selected his associates with skill and they relieved him of much trouble by taking part in the work of planning and keeping an eye on the implementation of plans.

In recognition of his scientific activities, in 1951 he was elected a corresponding member, and in1964 an ordinary member of the Hungarian Academy of Sciences. In 1956 he was awarded the Kossuth prize, in 1960 the Medal of Merits in Socialist Work, in 1963 the Order of Labour, in 1965 the gold medal of Eminent Inventor. The international reaction to his achievements in carbohydrate chemistry was reflected by his election into the editorial board of "Carbohydrate Research", an international journal started in 1964.

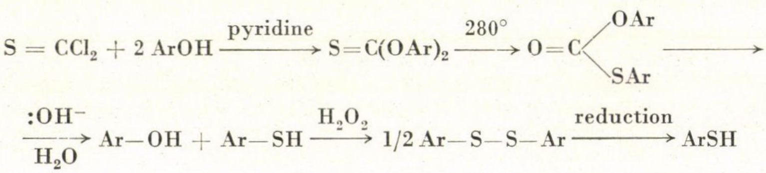
Vargha's scientific activity is evidenced by nearly 100 papers published mostly in international journals in German and English. In addition to this, he has worked out numerous pharmaceutical syntheses described in patents. His studies covered several branches of organic chemistry, though his attention was primarily attached to the chemistry of carbohydrates. Owing to the interesting results attained in this field, he commanded appreciation already as a young chemist even among foreign specialists. His scientific activity was characterized by the thorough knowledge of the relevant literature, an excellent sense of criticism and inventive thinking. Being a very good experimenter as well, in the later phases of his career when he was increasingly burdened with the tasks of organization and management and his physical condition — owing to his progressing disease — was gradually impaired, he did not readily acquiesce in the fact that his personal participation in the exciting process of performing laboratory experiments became more and more limited.

One of his early contributions to the chemistry of carbohydrates, made as a coworker of Ohle, is the synthesis of 1,2-0-isopropylidene-6-0-tosyl-D-glucofuranose (I), then from this — after Vargha's idea — with an equimolar amount of sodium methylate, the preparation of the 5,6-anhydro derivative (II). This was a new and simple method for producing sugar epoxides, successfully applied later by others too. From epoxide II, with alkali one obtains 1,2-0-*iso*-propylidene-D-glucofuranose (III). On the other hand, nucleophilic attack by the hydroxide ion at the carbon atom in position 5 yields the *iso*propylidene derivative of the rare D-idose (IV). It was thought first that, under the influence of excess sodium methylate, the epoxide II was converted to 1,2-0-isopropylidene-5-0-methyl-D-glucofuranose (V), the acid hydrolysis of which afforded 5-0-methyl-α-D-glucofuranose (VI). This would have been the first known crystalline D-glucofuranose derivative. Later, however, the *trans*-opening of the epoxide ring was shown to occur, leading, instead of the D-glucose derivative VI, probably to the L-idose derivative VIa:



The structure of the new sugar derivatives obtained and the number of atoms in the lactol ring have been rigorously proved by further transformation.

An interesting result of the studies carried out in collaboration with Schönberg [6—11] was the thermal isomerization of the aromatic thion esters of thiocarbonic acid into thiol esters. This led to a convenient method for converting phenols into thiophenols, which cannot be carried out in a satisfactory manner with phosphorus pentasulfide:



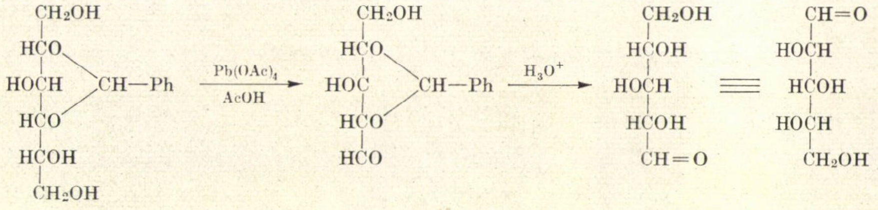
$$S = CCl_2 + 2\,ArOH \xrightarrow{\text{pyridine}} S{=}C(OAr)_2 \xrightarrow{280^\circ} O{=}C\begin{matrix}\diagup OAr \\ \diagdown SAr\end{matrix} \longrightarrow$$

$$\xrightarrow[H_2O]{:OH^-} Ar{-}OH + Ar{-}SH \xrightarrow{H_2O_2} 1/2\,Ar{-}S{-}S{-}Ar \xrightarrow{\text{reduction}} ArSH$$

Vargha's first independent research project at home — which he started at Szent-Györgyi's institute in Szeged — soon yielded important results. It was then that Szent-Györgyi isolated the so called "hexuronic acid" from red pepper juice with remarkable ease and in large quantities. This substance was first isolated by him several years earlier from adrenal cortex in a lengthy procedure. Because of its occurrence in plants and its reducing nature even in acidic media he considered this compound to be the elusive Vitamin C, an assumption which he supported by animal tests, too. However, several experts of vitamin research doubted the correctness of this statement, since the curative and preventive daily doses of hexuronic acid (1 mg/kg body weight) observed in the animal experiments were larger by 2—3 orders of magnitude than in the case of isolated, although not quite pure, vitamins. Thus his view that the hexuronic acid may contain Vitamin C only as an impurity was understandable. Then Vargha prepared an *iso*propylidene derivative of hexuronic acid which he repeatedly recrystallized, then regenerated from it the hexuronic acid [14]. According to animal tests, this compound was — also quantitatively — just as efficient as the initial hexuronic acid. Thus hexuronic acid was proved to be identical with Vitamin C. After the determination of its structure (1934), it was named L-ascorbic acid. The following two observations of Vargha provided a starting point for the determination of the structure: a) *iso*propylidene-ascorbic acid — like ascorbic acid itself — is a powerful reducing agent of acidic character which, when dissolved in water, decomposes into ascorbic acid and acetone [14]; b) ascorbic acid contains a primary alcoholic hydroxyl group that can be tritylated [15].

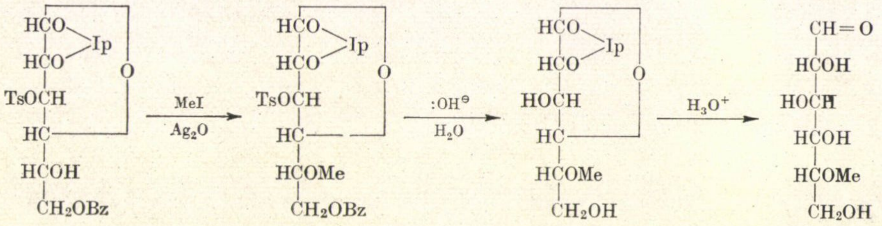
During the short time he worked at Tihany he elaborated an ingenious procedure for the simple synthesis of 1,2-0-isopropylidene-α-D-glucofuranose, which was based — instead of the partial hydrolysis of di*iso*propylidene-D-glucose — on the methanolic cleavage of 1,2-0-*iso*propylidene-α-D-glucofuranose-3,5-boric acid ester obtainable by the simultaneous reaction of acetone, boric acid and D-glucose [16]. He soon utilized this method in the synthesis of 1,2-0-*iso*propylidene-D-mannite too [17], and with this started a wide range of investigations on sugar alcohols. These studies proved to be very fruitful in his subsequent research in pharmaceutical chemistry.

After an erroneous first suggestion [21], he correctly recognized the structure of benzalsorbite [22], which had been described much earlier but its structure was unknown. He immediately utilized his idea in the synthesis of L-xylose (accessible before only with difficulty) through the route VII → VIII → IX. At the same time, using the pyridine technique, he isomerized L-xylose into L-xyloketose, a ketopentose which had been isolated by Levene and LaForge from urine as early as 1914, but only the synthesis of its D-antipode was known.



VII VIII IX IX

The synthesis of the previously unknown 5-0-methyl-D-glucose (XIII) from 1,2-0-*iso*propylidene-3-0-tosyl-6-benzoyl-α-D-glucofuranose (X) via the route X → XI → XII → → XIII [24] deserves special attention.

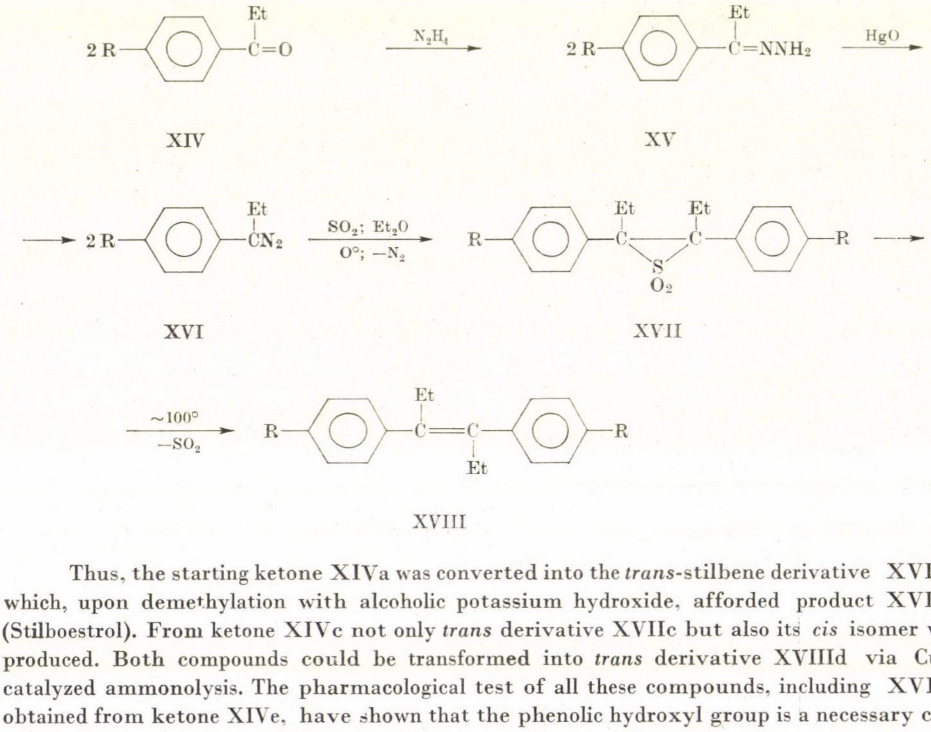


X XI XII XIII

It is the preciseness of the work rather than the route of the synthesis that is remarkable. He has rigorously proved the absence of acyl migration during the transformation XI → XII, prepared a crystalline derivative of the syrupy end-product and has demonstrated that the *al-*

D-glucose form predominates in the end-product. This in itself is a very important statement indicating the extreme lability of the D-glucofuranose ring.

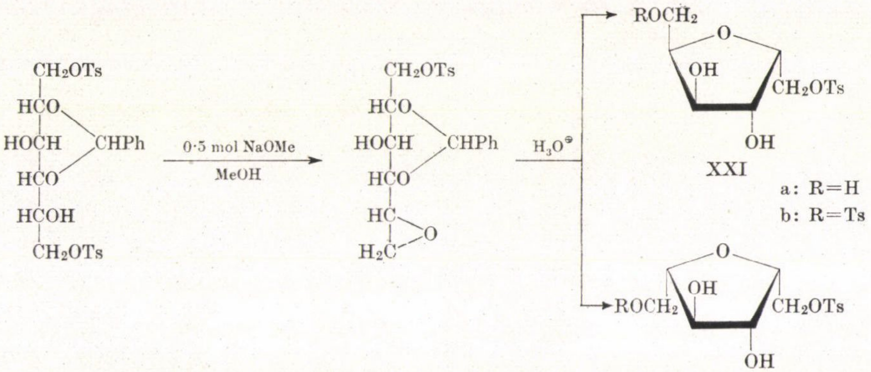
With this publication written in the first days of August, 1936, Vargha's research in the chemistry of carbohydrates was interrupted for a while, as he began his work in the Gedeon Richter Pharmaceutical Factory. Among his pharmacochemical studies performed or started there [25—28, 30]. the first to be mentioned is undoubtedly the ingenious synthesis of *trans*-1,2-diethyl-1,2-di-(*p*-hydroxyphenyl)-ethylene (XVIIIb). This artificial oestrogen compound was described by Dodds and Robinson in 1937; in 1938 its synthesis starting from anisaldehyde through anisoine, which demonstrated its structure, was also elaborated and patented. In the same year Vargha developed a much simpler synthesis with improved yield (date of patent application March 30, 1938). At the same time he synthesized derivatives containing other substituents instead of the two phenolic hydroxyls, in order to be able to study the relationship between biological effect and molecular structure. The starting material was propiophenone or its 2-substituted derivative (XIV) and the target product (XVIII) was obtained via the route XIV → XV → XVI → XVII → XVIII: *a:* R = OMe; *b:* R = OH; *c:* R = Br; *d:* R = NH_2; *e:* R = H



XIV XV XVI XVII XVIII

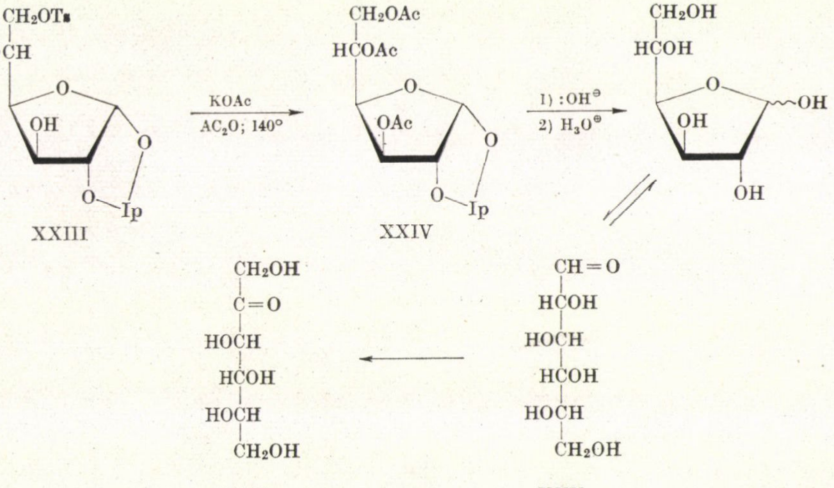
Thus, the starting ketone XIVa was converted into the *trans*-stilbene derivative XVIIIa which, upon demethylation with alcoholic potassium hydroxide, afforded product XVIIIb (Stilboestrol). From ketone XIVc not only *trans* derivative XVIIc but also its *cis* isomer was produced. Both compounds could be transformed into *trans* derivative XVIIId via Cu_2I_2 catalyzed ammonolysis. The pharmacological test of all these compounds, including XVIIIe obtained from ketone XIVe, have shown that the phenolic hydroxyl group is a necessary condition of the oestrogen activity.

While a professor in Kolozsvár he carried on his studies on carbohydrates, but at the same time he maintained close relations with the Hungarian pharmaceutical industry. He continued [29] his earlier work [21, 22] on benzylidenesorbite, studying the hydrolysis in dilute acid of 1-0-tosyl-2,4-0-benzylidene-5,6-anhydro-D-glucite (XX) previously obtained from 2,4-0-benzylidene-1,6-di-0-tosyl-D-glucite (XIX) by partial detosylation (sodium methylate, MeOH). This reaction did not lead to a 1-0-tosylhexite (a D-glucite and/or D-idite derivative could be expected) but to a 1-0-tosyl-X,X'-anhydrohexite, which was shown to contain two primary and two secondary vicinal hydroxyls in *trans* position. This property may be due to the 1-tosyl derivative of both 2,4-anhydro-L-idite and 2,4-anhydro-D-glucite (XXIa, XXIIa). A decision was reached in favour of XXIa since the oxidation of the corresponding ditosylate with lead tetraacetate yielded an optically active dialdehyde. The dialdehyde originating from the analogous D-glucite derivative (XXIIb), being of the *mezo* configuration, would have been optically inactive.



XIX XX XXII

Vargha's attention was often attracted by the isomerization and epimerization of sugars and sugar alcohols [39] which is understandable since the most convenient way of epimerization is through the epoxides, and the latter can be prepared by the easily generalized method of Vargha and Ohle [6]. This has turned his attention towards the 0-tosyl and 0-mesyl derivatives. One of his results in this field is the relatively simple synthesis of L-idose from D-glucose in good yield [41] via the following route. The tosyloxy groups of 1,2-0-*iso*-propylidene-5,6-di-0-tosyl-α-D-glucofuranose (XXIII), readily obtained from D-glucose, can be substituted by acetoxy groups via an SN_2 reaction involving the inversion of the asymmetry centre at position 5. The alkaline then acid hydrolysis of the triacetate obtained in this way (XXIV) yields L-idose:



XXVI XXV

According to chromatographic evidence, the freshly isolated syrup of L-idose is a pure compound. However, after standing for a week it is found to contain L-sorbose (XXVI). The reaction product becomes increasingly turbid and, after 3 months, nearly 60% of crystalline L-sorbose (XXVI) can be isolated. Thus the L-idose (which, according to its reactions, is present in the equilibrium mixture mainly as the *al*-form) is labile, in line with the fact that neither this nor its D-antipode occur in nature. Its transformation into L-sorbose can be attributed to stereochemical causes [41].

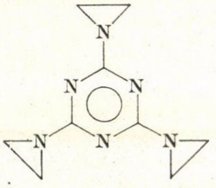
The work discussed above was Vargha's first publication in the field of carbohydrate chemistry submitted from the Pharmaceutical Research Institute; it was soon followed by numerous papers on the same subject. As a consequence the Pharmaceutical Research Institute has become an important base and, at the same time, school of Hungarian carbohydrate research. Numerous young researchers were introduced under Vargha's guidance to this important field of organic chemistry and have become internationally known carbohydrate chemists.

The first impulse to initiate studies in this field in the Pharmaceutical Research Institute was given by Vargha's idea that the known cytostatics paralyzing cell division, and used to fight cancer, are mainly derivatives of substances rejected by the body, and this may be the very reason that their effect is not selective, that is, they destroy not only the cancerous but also the normal cells, and it is from this that their high toxicity originates. These considerations motivated his review article written in collaboration with six of his associates, published in 1961 [66]. This publication summarized the results of research carried on for ten years in the Pharmaceutical Research Institute with the object of synthesizing sugar and sugar alcohol derivatives containing cytoactive groups [47—49, 55, 56, 59, 65]. This work was continued also after 1961 as shown by a considerable number of papers on the subject [71—78, 81, 82, 84, 86—88, 93—96]. The studies involved a great deal of basic research on carbohydrates, since Vargha, on the basis of his great experience was deeply convinced that pharmacochemical research is closely connected with basic research in organic chemistry. The large number of publications originating from the Pharmaceutical Research Institute were written in this spirit, this laid the foundation of the high international reputation of the Institute, and it was due to this spirit that several members of this institute rose to the first ranks of Hungarian organic chemists.

Among the cytoactive groups Vargha's attention was primarily attracted by the biological alkylating agents whose prototype was regarded to be the so called nitrogen mustard (XXVII), though 2,4,6-triethyleneimino-*sym*-triazine (XXVIII) and 1,4-dimesyloxybutane (XXIX) may also be classed into this group.

$CH_3N(CH_2CH_2Cl)_2$

XXVII

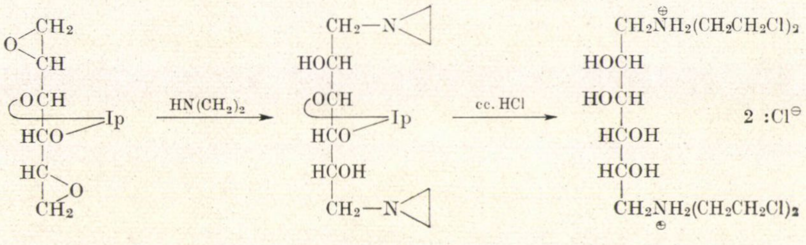


XXVIII

$CH_3SO_2OCH_2CH_2CH_2CH_2OSO_2CH_3$

XXIX

According to literature data, the nitrogen mustard analogues containing various other groups instead of the methyl group do not surpass the biological effect of nitrogen mustard, nor is their toxicity lower. There was a hopeful possibility that the effect of a cytoactive (alkylating) group attached to carriers non-alien to the cell could be made less toxic and thus more selective. On this basis two previously unknown sugar alcohol derivatives (XXXI and XXXII) containing cytoactive groups were prepared from 1,2-5,6-dianhydro-3,4-0-*iso*propylidene-D-mannite (XXX) via the route XXX → XXXI → XXXII. Derivative XXXII had a therapeutic index superior to that of nitrogen mustard and, therefore, was soon introduced into medical practice under the name "Degranol".


XXX XXXI XXXII

This result contributed to recognizing the facts that a) the two "alkylating" groups need not necessarily be attached (as in the nitrogen mustard) to the same N-atom to attain a cytostatic effect; b) the hydroxyl groups undoubtedly contribute to the favourable therapeutic index, since the hydrochloride of the 1,6-di-(β-chloroethylamino)-hexane synthesized for comparison proved to be completely ineffective. On the basis of this realization extensive studies were started at the Pharmaceutical Research Institute; numerous sugar and sugar alcohol derivatives containing cytoactive groups were prepared mainly with the view of studying the relationship between the structure of the carrier molecule and its cytostatic effect and therapeutic index. In addition to sugar derivatives containing the cytoactive chloroethylamino group, derivatives with ethyleneimino and mesyloxy groups as well as α,ω-dideoxy-α,ω-dihalogeno sugar alcohols were also prepared. On the basis of the known cytostatic effect of the natural antibiotic azaserine, diazo- and bis-diazo sugar derivatives were synthesized.

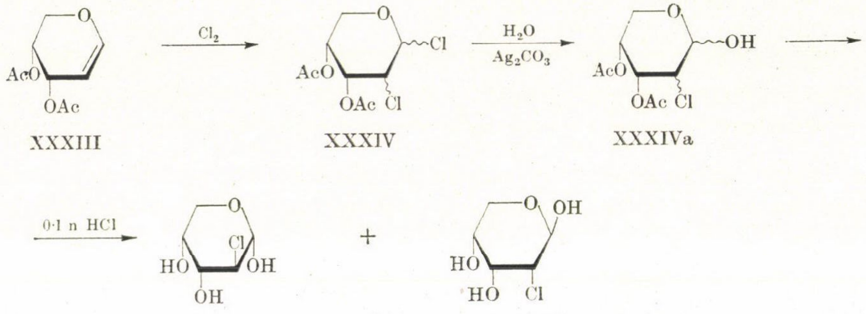
In accordance with this objective, the L-mannite, D-glucite, dulcite and L-idite derivatives analogous to Degranol were first synthesized in order to study the relation between asymmetric centres and biological effect; on the basis of their pharmacological tests the importance of the chirality of the asymmetry centres became apparent, and at the same time the therapeutic index was found to be the most favourable in the case of the D-mannite derivative which had been the first to be prepared (XXXII). The halogen atoms of XXXII were also varied and the corresponding bromine derivative was found to have a more favourable therapeutic index than Degranol. This is in line with the assumption that the effect of biological alkylating agents is due to their ability to form cross-links between protein (enzyme) chains and nucleic acids by bonding to nucleophilic centres via their substituted alkyl groups.

The aldonic acid-β-chloroethylamides prepared from the corresponding aldonic acid lactone and saccharic acid dilactone, as well as saccharic acid and its D-manno-di-β-chloroethylamide proved to be ineffective. A thorough knowledge of the carbohydrates and a great ingenuity are reflected by the syntheses which resulted in a number of sugar derivatives containing a cytoactive centre truly analogous with the nitrogen mustard ((N,N-di-β-chloroethyl-D-glucoseamine and its L-antipode; 5-deoxy-5-(di-β-chloroethyl)-aminoethyl-D-ribofuranoside; 1-deoxy-1-(di-β-chloroethyl)-amino-2,3-*iso*propylidene-D-fructofuranoside; 1-deoxy-1-(di-β-chloroethyl)-amino-2,3-*iso*propylidene-L-sorbofuranose)) and the salts of the basic compounds listed. Nevertheless, all these compounds showed a much lower cytoactivity than Degranol (XXXII) and were also highly toxic.

Finally, Vargha extended his attention to the dimesylates, another group of biological alkylating agents, since 1,4-butanediol dimesylate (XXIX) was found to be a cytoactive compound, and so the extension of the carbon chain and the simultaneous incorporation of hydroxyl groups were expected to reduce the toxicity. It was hoped that the variation of the chirality of asymmetric centres would lead to compounds of high selectivity. This idea led to the synthesis of 1,6-di-0-mesyl-D-mannite and its L-antipode, of 1,6-di-0-mesyl-L-sorbose, 1,6-di-0-mesyl-D-fructose and 1,4-di-0-mesyl-*meso*-erythrite. Of these dimesylates it was again the D-mannite derivative ("Mannogrand") that proved to have the most favourable effect, calling attention again to the importance of the length of the carbon chain and the chirality of the asymmetry centres.

With reference to the cytostatic effect of azaserine, from the appropriate aldonic acid and saccharic acid chloride, respectively, 1-diazo-1deoxy-D-glucoheptulose and the 1,8-bis-diazo-2,4-dioxo-3,4,5,6-galactotetrahydroxyoctane were synthesized with diazomethane; however, these sugar derivatives did not even attain the cytoactivity of Degranol (XXXII).

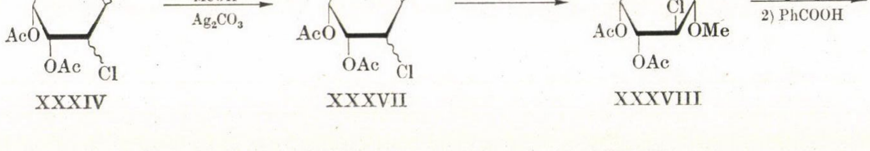
During the above investigations Vargha's attention turned toward another principle of the chemotherapy of cancer: antimetabolites with purine and pyridine skeletons. The first publication in this subject [73] describes the synthesis of 2-chloro-2-deoxy-D-ribose and -D-arabinose (XXXV and XXXVI). The reason for choosing these two compounds was that the hydroxyls at C-3 and C-5 and the appropriate configuration of asymmetry centre No. 3 required for phosphorylation and thus for incorporation into nucleic acids, were invariably present. For the synthesis of these two compounds the starting material was the easily available diacetyl-D-arabinal (XXXIII), from which the mixed product XXXVI was obtained by chlorine addition. By two-stage hydrolysis the desired D-arabinose was obtained on one hand, and a D-ribose derivative on the other (XXXV, XXXVI); these two crystalline products were separated and their structures rigorously proved.



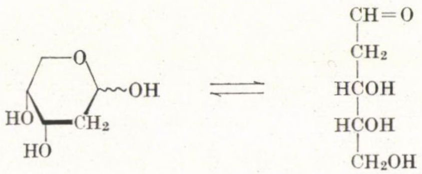
XXXIII XXXIV XXXIVa

XXXV XXXVI

Dichloro derivative XXXIV permitted to develop a new synthesis with higher yield for the important 2-deoxy-D-ribose. In the presence of silver carbonate and methanol a mixture of the methyl glycosides XXXVII was formed from product XXXIV, from which the D-arabinose derivative (XXXVIII) could be isolated in a crystalline form. Its hydrogenolysis performed in alkaline medium with Raney nickel followed by hydrolysis with 2% benzoic acid gave 2-deoxy-D-ribose (XXXIX) [73].

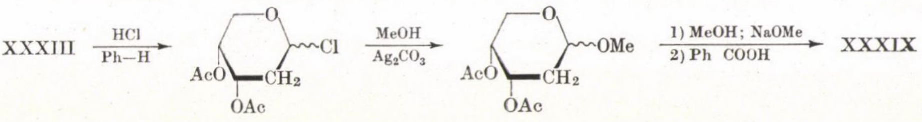


XXXIV XXXVII XXXVIII



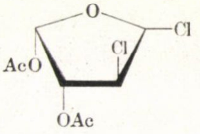
XXXIX

This biologically important aldose was prepared already in 1949 from diacetyl-D-arabinal (XXXIII) with the old Fischer method (water added in acidic medium), however, the yield was as low as 10%, while with the new technique 65% could be attained. A further simplification of the new method and improvement of the yield was attained in the following way: the addition of HCl to diacetyl-D-arabinal (XXXIII) in benzene solution afforded 2-deoxy-acetyl-chloro-D-ribopyranose (XL). The methyl glycoside produced from this (XLI) by Zemplén's deacetylation method was carefully hydrolyzed with acid to yield 80% of 2-deoxy-D-ribose crystallized in the form of its anilide (XXXIX) [74].

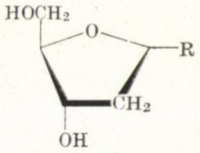


XL XLI

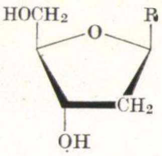
The first efforts to couple the well-defined, crystalline 2-deoxy-2-chloro-D-arabinose with adenine to obtain the corresponding 2'-chloro-adenosine were unsuccessful, although one of the components, 1,2-dichloro-3,5-0-diacetyl-1,2-dideoxy-D-arabofuranose (XLII), required to form the N-glycoside was successfully produced from the appropriate pyranose (XXXIVa). Since in the glycoside coupling, adenine was used as the sodium salt of 2,8-dichloro-adenine, the selective removal of the two aromatic chlorine atoms failed. However, the reductive elimination of all three chlorine atoms in an alkaline medium resulted in the formation of 2'-deoxy-α- and β-D-ribofuranosidoadenine (XLIII and XLIV), which in itself is a valuable achievement:



XLII



XLIII



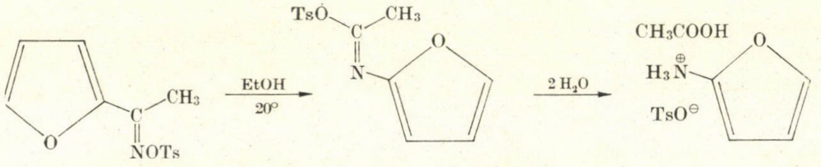
XLIV

The desired 9-(2'-chloro-2'-deoxy-α-D-arabofuranosido)-adenine and its β-anomer were obtained finally through the reaction of 6-benzoylamino-9-chloromercurypurine with derivative XLII, followed by deacetylation of the product according to Zemplén's method [81]. Although both compounds showed but a low antitumour activity, their synthesis contributed new information to various aspects of organic chemistry.

Studies on the synthesis of given sugar and sugar alcohol derivatives often caused surprise but at the Pharmaceutical Research Institute Vargha and his coworkers skilfully solved the structural mysteries of unexpected compounds and sometimes corrected the literature data (e.g.[84]). And even if e.g. the cyclic esters of sugars and sugar alcohols formed with phosphoric acid di-β-chloromethylamide [86], or the diepoxides [87] or diepithio derivatives [88, 92] of sugars and sugar alcohols were of little value from a therapeutic point of view, their synthesis and the exact determination of their structure contributed important new discoveries to the chemistry of carbohydrates.

A few other results of the extensive studies concerned with the synthesis of suitable cytostatics have to be mentioned here. From D- and L-mannite, 2,3-4,5-dianhydro-1,6-di-0-mesyl-L-, and -D-idite, respectively [94], the 2,3-4,5-dianhydro-1,6-dideoxy-1,6-dibromo-L-idite [95], as well as the 1-bromo-6-chloro and 1-bromo-6-iodo derivatives of 1,6-dideoxy-D-mannite [96] have been prepared. In the syntheses of the above compounds interesting stereochemical problems were encountered and the ingenious detection of numerous intermediates gave evidence of excellent inventiveness. Apart from this the biological properties of all these compounds provided important data on the relationship between cytostatic effect and molecular structure, witnessing the high level of pharmacochemical research pursued at the Pharmaceutical Research Institute.

Investigations made on furan compounds are especially interesting [33—38, 52—54, 58, 79, 80, 97, 98]. They were started at Kolozsvár, originally with the aim of producing the previously unknown 2-amino-furan from 2-acetofuranoxime tosylate (XLV) with the so called Neher reaction via the route XLV → XLVI → XLVII, in order to study its biological effect.



XLV XLVI XLVII

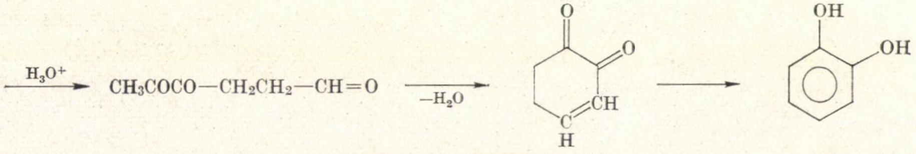
However, instead of the expected path, the reaction took place in another direction: beside the ammonium tosylate, a nitrogen-free, air-sensitive oil, distillable in vacuum, was produced which, according to elemental analysis, molecular weight and its chemical reactions, seemed to be hexenedional diethyl acetal (XLVIIIa); with methyl alcohol the expected dimethyl acetal (XLVIIIb) was obtained. The reaction can be written in the following way:

$$\text{XLV} + 2\,\text{ROH} + H_2O \rightarrow \text{TsONH}_4 + CH_3COCO{-}CH{=}CH{-}CH(OR)_2$$

$$\text{XLVIII a: R=Et; b: R=Me}$$

Since the unsaturated acetal, when oxidized with hydrogen peroxide in an acidic medium, yielded, among others, maleic acid, it was concluded that, of the two ethylene isomers, the *cis* form predominated. As the hydrolysis of the acetal with dilute acid involves extensive decompositions, the diketohexane-al (L) seemed to be attainable by the acid hydrolysis of the derivative saturated by catalytic hydrogenation (XLIX); however, owing to an intramolecular condensation occurring instead, pyrocatechol (LI) was formed, which proved again the correctness of formula XLVIII:

$$\underset{\text{XLVIII}}{CH_3COCOCH{=}CH{-}CH(OR)_2} \xrightarrow{Pt/H_2} \underset{\text{XLIX}}{CH_3COCOCH_2CH_2CH(OR)_2} \rightarrow$$

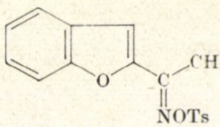


L LI

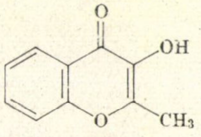
The interesting result, revealing the connection between pyrocatechol and acetofuran, points to a possible biogenetic relation between natural benzene and furan derivatives (e.g. furanoses).

Although from oxime tosylate XLV, the desired 2-aminofurane could not be obtained, the alternative reaction observed appeared to be novel and interesting so that Vargha and his coworkers extended the studies to the oxime tosylates of other furan ketones too. Among others they studied the transformation of 2-acetocoumarone, 2-propiofuran and 2-benzoylfuran oxime tosylates in the presence of ethanol and methanol. These compounds underwent a variety of transformations. For example, the 2-acetocoumarone oxime tosylate (LII), in addition to ammonium tosylate, produced two crystalline and one oily products distillable in vacuum. One of the former products proved to be the previously unknown 2-methyl-3-hydroxychromone (LIII), the other was tentatively assumed to have the structure of chromanone derivative LIV.

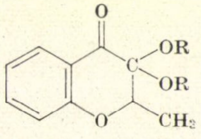
The formation of various products called attention to the study of the configuration of oxime tosylates, since the two geometric isomers were expected to show different behaviour during alcoholysis. Therefore, thorough investigations were started with the aim of producing and identifying the *syn*- and *anti*-modifications of certain ketoximes. This effort was successful and members of six ketoxime isomer pairs containing 2-furyl or 2-benzofuryl groups were prepared and identified, correcting at the same time some literature data [52]. The reaction of the tosylates of these compounds with alcohols was found to depend on the configuration: the *syn*-furyl-2-alkylketoxime tosylates (LV) did not change under the influence of cold ethanol or methanol; the *syn*-furyl-2-arylketoxime tosylates (LVI) underwent Beckmann rearrangement; while the above mentioned unsaturated acetals were obtained only through *anti*furyl derivatives (LVII) with a single exception: from *anti*-5-methylfuryl-2-methyl ketoxime tosylate (LVIII), levulinic acid was formed via Beckmann rearrangement.



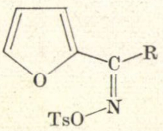
LII



LIII

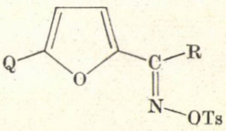


LIV



LV R=Alkyl

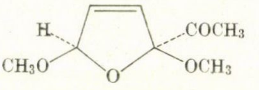
LVI R=Aryl



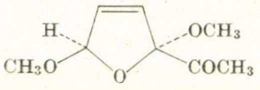
LVII Q=H

LVIII Q=CH₃

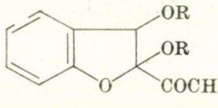
At the beginning Vargha and his coworkers did not attempt to interpret the mechanism of these unusual transformations since they made conclusions on the structure of unsaturated acetal XLVIII only from its chemical reactions and oxidative decomposition, and did not succeed in implementing the synthesis to demonstrate the structure. However, Dunlop and Peters in 1957, and Meinwald in 1958 attempted to interpret the mechanism of this interesting reaction. With the two mechanisms, not differing essentially from one another, the course of the reactions and their distinctive features in the case of *syn-* and *anti*-oxime tosylates could be satisfactorily interpreted with the steric factors taken also into consideration. However, in 1967 Greene and Lewis reproduced the alcoholysis of *anti*-oxime tosylates of the type LVII and on the basis of IR, UV and NMR spectroscopic studies of the unsaturated acetals obtained, pointed out that they were not open-chain compounds but the derivatives of 2,5-dihydrofuran. Thus, e.g. the structure of the product obtained from 2-aceto-furan-*anti*-oxime tosylate (LVII; $R = CH_3$) and methanol was not of the type XLVIII but of the isomeric LIX, which could be separated by gas chromatography into *cis-* and *trans*-components (LIXa, LIXb).



LIXa



LIXb



LX

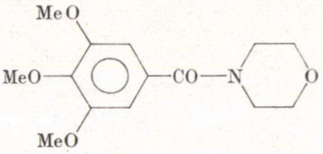
Otherwise structure LIX is also in agreement with all of the chemical reactions which seem to support structure XLVIII.

Finally, the structure of the chromanone derivative LIV seemed still to be questionable. Recently Vargha and his coworkers have identified this compound by IR, NMR and mass spectroscopic analyses as a coumarane derivative (LX), and — at the same time — modified the former conception concerning the mechanism of the alcoholysis of *anti*-oxime tosylates according to the new structural information [98]. In this way a satisfactory explanation was given for the reaction of *anti*-oxime tosylate LII with alcohol containing some water, yielding 2-methyl-3-hydroxychromone (LIII) and chromanon derivative LX. The latter was also separated by gas chromatography into *cis* and *trans* isomers. The methanolysis of the tosylate of 2-acetofuran-*anti*-oxime leading to the formation of 2,5-dihydrofuran derivatives (LIX) could be explained in the same way. All of these reactions start with nucleophilic alcohol addition running parallel to the displacement of the tosylate anion and occurring at C-5 in the furan skeleton and C-3 in the benzofuran skeleton.

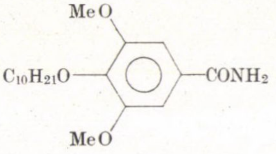
The condition for nucleophilic attack is the *anti*-position of the tosyloxy group relative to the furan ring, since only this ensures the removal of the tosylate anion owing to coplanarity and the shift of the positive charge to C-5 in the furan ring, and to C-3 in the benzofuran ring. In the latter case the aromatic stability of the benzene ring due to its π-electron sextet inhibits the shift of charge to carbon atom No. 5 in the condensed furan skeleton.

The transformations of furylketoxime tosylates were found later to have pharmacochemical consequences, too. On the basis of the known vasodilatory effect of khellin, containing the furanochromone skeleton, Schmutz and coworkers, studied in 1951 the effect of numerous simpler chromone derivatives including 2-methyl-3-hydroxychromone (LIII) prepared by Vargha and his coworkers from the tosylate LII, and independently synthesized later, and found that their effect reached and occasionally even surpassed that of khellin. Since khellin contains two methoxy groups, several dimetoxy-2-methyl-chromones and dimetoxychromone-2-carboxylic acid esters were synthesized at the Pharmaceutical Research Institute to study the pharmacological effect and the molecular structure [40]. These compounds were found to be more selective coronary dilators and their effect was five to six times higher than that of khellin, whose isolation and synthesis are rather difficult.

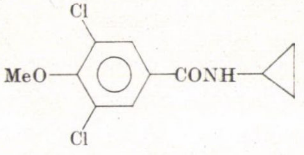
The benzoyl amide derivatives substituted by cyclic ether groups, or ether and hydroxyl groups, are of considerable interest. A study was started because numerous alkaloids were known to contain ether groups bonded to an aromatic ring (e.g. MeO-, CH_2O_2= groups), so it did not seem impossible that simpler compounds would show some biological effect too. As many as 50 compounds, mostly unknown before, were synthesized of which 2,3,4-trimethoxybenzoyl morpholide (LXI) excelled with its tranquillizing effect and low toxicity [69, 70]. This gave the impulse to prepare various compounds containing substituted phenyl groups [91] and study their effect on the contral nervous system [92]. Among them, some dibromo derivatives displayed a significant *anti*-nicotin effect and, with them in possession, a study on the relationship between *anti*-nicotin effect and molecular structure became possible. This group includes the highly spasmolytic 2,5-dimethoxy-4-decyloxybenzoic acid amide (LXII), and the narcotic 3,5-dichloro-4-methoxybenzoic acid cyclopropylamide (LXIII):



LXI



LXII



LXIII

Besides publications reporting on syntheses aimed at the discovery of new antituberculotics [42—44], the essential improvement of Wibaut's method for the preparation of 4-ethylpyridine, the starting material of the production of isonicotinic acid hydrazide deserves special mention; it simply consists in employing iron instead of zinc in the reaction of acetic anhydride with pyridine, by which the yield is doubled [51].

Vargha's many-sided research in organic and pharmaceutical chemistry is best demonstrated by the essential improvement of the isolation of hyodeoxycholic acid from pig gall. This substance was then used to prepare 3β-acetoxy-5-cholic acid methyl ester, a suitable starting material for the semi-synthesis of steroid hormones. Although the latter product did not give a satisfactory yield, this work deserves attention because of its stereochemical implications [46].

It is hoped that the present survey has succeeded in showing Vargha's quality as a researcher, his wide knowledge and excellent achievements both in pharmaceutical and organic chemistry. As Director of the Pharmaceutical Research Institute, he always endeavoured to support pharmaceutical research with basic research in organic chemistry, since — in his opinion — this is the only way towards the discovery of new medicines, which is a necessary requirement to ensure continued development of the Hungarian pharmaceutical industry. Guided by this view, he greatly promoted the relations of the Pharmaceutical Research Institute with university institutions and with the Hungarian pharmaceutical industry. He initiated numerous research projects and promoted ones initiated by others if he was convinced of their importance. It is for this reason that at the Pharmaceutical Research Institute basic research in organic chemistry was pursued on a wide range of subjects, and that many of his coworkers have become excellent research chemists. Some of them have obtained scientific degrees on the basis of their work done at the Institute, and six have been awarded Kossuth and State prizes. Rightly was his Institute the first among research institutes supervised by the Ministry of Heavy Industry to receive the honourable title of "Eminent Research Institute". His ten years of activity as a professor at Kolozsvár and the efficiency of his teaching and educational work is testified by the success of his students as teachers or chemists.

In spite of the great stress of his work, Vargha was outstandingly active in scientific organizations too. As a member of the Department of Chemical Sciences of the Hungarian Academy of Sciences, as well as member of the Committee on Organic Chemistry belonging to the Department, and recently as chairman of the Committee on Carbohydrate Chemistry he often voiced his views and was an outspoken critic. As president of the section of organic chemistry in the Hungarian Chemical Society he was an untiring organizer of Hungarian conferences on organic chemistry. He laid great emphasis on maintaining international scientific relations; he knew personally many excellent foreign scientists and had many friends among them.

His health gradually deteriorated in the last years; his illness, which became more and more serious, required an almost superhuman effort to carry out his assignments, many of them voluntary. This acceptance of responsibility and the love of scientific research gave him the strength to stay at his post to the last. With his death, organic chemical and pharmacochemical research has been deprived of an outstanding and internationally acknowledged expert. His life has been exemplary, and the achievements and concepts of his extensive work will keep his memory alive.

V. Bruckner

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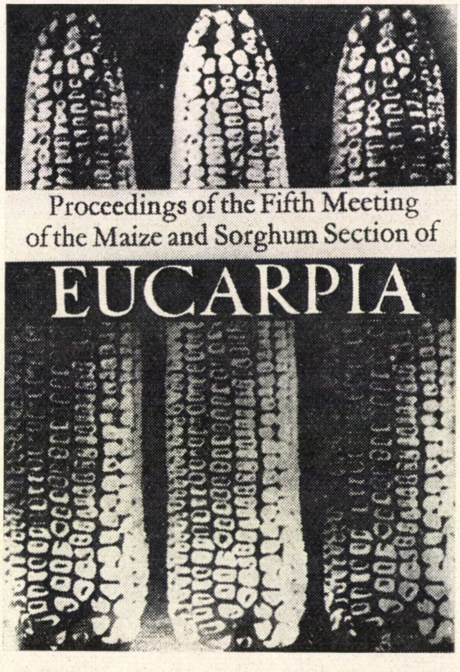
RECENSIONES



Proceedings of the Fifth Meeting of the Maize and Sorghum Section of EUCARPIA. Akadémiai Kiadó, Budapest 1971, 290 p. 100 tables, 34 figures. Ed.: I. Kovács.

The well got-up book contains the lectures delivered at the meeting of the Maize and Sorghum Section of EUCARPIA (European Association for Research on Plant Breeding) held in Budapest—Martonvásár during September 2—4th, 1969. The book published in 1971 does not mention, however, the date of the meeting, which may cause misunderstanding, as the Section held a meeting (the sixth) in 1971 too, in Freising—Weihenstephan. On the other hand, it is not quite the same for the authors whether their papers are read as lectures delivered in 1969 or as ones delivered in 1971; namely, as a result of the rapid development of science, it may happen that a paper containing very interesting new results in 1969 would be in 1971 the repetition of generally known facts. Thus, the question may arise, whether it had not been more reasonable to publish the papers earlier, by duplicating them with an up-to-date method instead of the unobjectionably got up book. This delay, however, also offered an opportunity to complete the material here and there with 1970 data (see p. 156; the year 1970 — instead of 1967 — is a misprint on page 172. No errata is annexed; however, the number of misprints is very few and only one or two of them may distort the sense).

On the basis of their subjects the forty lectures delivered at the meeting can be divided into seven groups.

Quality improvement with regards to proteins was introduced by the excellent lecture of D. E. Alexander (Illinois USA), which not only gave a complete survey of the results attained with the opaque-2 gene, but also discussed the future possibilities. The lecture emphasized the importance of tryptophane beside lysine, and presented economic calculations concerning the extent to which the yield decreases involved with proteins of higher value were still economical, while at the same time breeding work also showed promise that yield decreases involved with more valuable proteins would be avoidable.

M. Pollacsek first gave an account of the results obtained with opaque-2 breeding carried on in Clermont-Ferrand (France): the lower thousand-grain-weight of opaque-2 hybrids resulted in a considerable yield decrease. In his second lecture he pointed out on the basis of experiments that the effect of the opaque-2 gene resulting in an increased lysine content can be separated to some extent from the floury structure of the grain.

S. Rautou (Montpellier, France) presented a quick method for the evaluation of the lysine content: accordingly, breeders, when preselecting — are not compelled to rely exclusively on the floury character of the grains, whereby the combinations coupling the high lysine content with a certain degree of vitreosity are discarded.

Bálint A. first reported on the results of crossings carried out in Gödöllő (Hungary) in order to increase the protein content, then, in his second lecture, on the protein content of mutants produced by irradiation.

Kovács I. et al. presented the results of opaque-2 and floury-2 breeding carried out at Martonvásár (Hungary), specifying 17 different amino acids in the various groups of crosses, for the whole grain and the endosperm separately.

M. S. Mišović (Zemun Polje, Yugoslavia) pointed out by rat feeding experiments that maize containing opaque-2 gene — though lagging behind maize completed with soybean meal — produced a weight gain twice as much as normal maize did.

The first of the lectures discussing the oil content of maize was again by D. E. Alexander on his successful work carried on in Illinois (USA): selection for oil content started in 1896 was completed from 1940 on with hybrid breeding methods, and the most recent high oil content hybrids (IHO = Illinois High Oil) combined the more than 8 per cent oil content with good productivity similar to that in commercial hybrids. He discussed the prospects before high oil content maize due to the one-grain oil test that does not harm the germinability, and the new oil quality test that can be performed within a few seconds from the point of view of rentability too.

In the second brief lecture dealing with oil content M. S. Mišović (Zemun Polje, Yugoslavia) et al. pointed out experimentally, by a detailed analysis of their high oil content lines that no correlation exists between the oil content and the ratio of various fatty acids that might possibly prevent the combination of quantity and quality.

The last two lectures on breeding for quality dealt with the carotene content. M. Mihajlović et al. when determining the β-carotene- and cryptoxanthin contents of 30 hybrids found a ratio of 8 : 5 between the two (as to the closeness of the correlation no correlation coefficient was given). Between the individual hybrids remarkably great differences were found. Since the extreme values are presented, the regrettable omission of the table referred to in the text may disturb only those interested in details.

Finally I. Cabulea (Torda, Roumania) reported on his research work carried on in the research institute of Minoprio (Italy) on the subject of carotene metabolism.

The second group of lectures dealt with the importance of breeding local varieties. J. Pavličić (Zemun Polje, Yugoslavia) was the first to present detailed results on the local variety research started by the EUCARPIA, while giving an interesting comparison of highly similar types of Yugoslavian, Italian and Roumanian local varieties. Their common origin is supported by the fact that the Roumanian local varieties are closer to local varieties in Yugoslavia than to those in Italy. The similarity of certain types of various countries was demonstrated by 11 photos, and 14 morphological and phenological data of 38 local varieties were presented. He did not explain, however, his grading of the "ear form" between the extreme values of 5.0 and 18.0 although it cannot be considered as generally known.

After his first lecture on the basic principles of the EUCARPIA's work of collecting, maintaining and classifying the local varieties A. Brandolini (Minoprio, Italy) discussed the effect of the Bolivian germ plasm in South-European maize breeding, while giving in detail the ear-, grain- and tassel measurements of 44 Bolivian local varieties, and the phenological and morphological data under three different climates of 32 of them. In addition, he studied the combining ability of local varieties with a more than usual intensity, and presented the number of chromosome knobs too in 23 local varieties.

The next five lectures dealt with the subject of cytoplasmic male sterility. E. J. Vakhrusheva (Krasnodar, Soviet Union) compared Texas (T)-type and Moldavian (M)-type male sterility, and — while enumerating several disadvantages of the former — drew the conclusion that both types should be used, and some questions concerning the inheritance of male sterility and restoration of fertility, are still to be further studied.

In the next lecture G. S. Galeyev (Kuban, Soviet Union) described those methods of sterile hybrid seed production in which seed production for a single paternal cross was also made on a sterile base. In this way detasseling by hand was perfectly eliminated and on the market corn producing field of double crosses the number of pollen shedding plants was over 96 per cent.

D. F. Alyev (Baku, Soviet Union) in his brief lecture on the effect of environmental conditions on cytoplasmic male sterility pointed out that under wetter and cooler climatic conditions sterility is less stable, especially in the case of the Moldavian type.

The fourth group of lectures, which dealt with the methods of breeding, was introduced by an interesting lecture in which A. Tavčar (Zagreb, Yugoslavia) gave detailed information on the root examination method of 3 weeks old seedlings. The method renders it possible for the breeder to obtain informatory data on the prospective extent of the heterosis effect in the F_1 generation by examining plants as early as at the age of three weeks. The next lecture delivered by B. Guryev (Kharkov, Soviet Union) reported on early breeding in the Ukraine, emphasizing the usefulness of early × late and flint × dent crossings. He recommended early lines and their crosses to be used as initial material for new early lines rather than early varieties, owing to the very low productivity of lines originating from the latter. J. S. Bojanowski (Warsaw) gave an account of Polish–Hungarian co-operation in maize breeding. Co-operation is necessary because seed for the large area of Polish silo maize production cannot be produced in Poland due to its late ripening, while the imported seed of foreign hybrids is not quite suitable for the Polish conditions.

Szopkó M. et al. (Szeged, Hungary) presented detailed data on their work in which they used the prolific character of plants to increase productivity. Finally, D. Fellner (England) discussed the introduction of grain maize in England, and F. Mikuž (Ljubljana, Yugoslavia) reported briefly on maize breeding in Ljubljana.

The fifth group of lectures dealt with breeding for resistance. Manninger I. (Martonvásár, Hungary) spoke about the considerable differences in resistance between sublines started from earlier established lines.

V. Spehar (Zagreb, Yugoslavia) studied resistance to *Helminthosporium turcicum* in a great number of lines and hybrids, describing the method of provocative infection. He touched upon the heredity of very high differences in resistance.

T. Perju (Kolozsvár, Roumania) gave a detailed account of the extreme damage done by frit-fly to maize in Transylvania, including differences between varieties too.

V. Penčić (Zemun Polje, Yugoslavia) presented an exhaustive report on the occurrence of *Sclerotium bataticola* Taub. root and stem rot in Yugoslavia, including a description of the fungus and its damages and the methods of control.

The last group of lectures dealing with maize, which covered various production and physiological problems, was introduced by a report on J. Gotlin's (Zagreb, Yugoslavia) spacing experiments. In the extremely precise experiment carried on over two years with 13—16 hybrids and four different spacings data on seven parameters — besides the yield — were obtained. The relatively few comments added to the data did not emphasize the great difference in weather conditions — and consequently yield averages — between the two years of the experiment. In 1967 — the year with the lower yield — more than one parameter showed different trends, and even the optimum yield was obtained with less dense plant stands. So the statement that closer spacing increased the water content of grains and the number of plants increased from 50.000 to 60.000 per ha did not give significant differences should not have been generalized, as the yield decrease in four late hybrids under the influence of such a close spacing was highly significant in 1967. Attention is called to the harmful effects of too close a spacing due to the intensive lodging. The trend of ear weight giving two-peak curves in several hybrids due supposedly to changes in the number of ears per plant (no parameter is presented) is very interesting.

The next lecture delivered by J. Gotlin dealt with the influence of eight different chemicals on maize germinated at low temperatures, presenting not only the germination percentage but four other parameters too. In this way not only the fungicide effect of the chemicals but also their stimulation of metabolism could be proved. Germination was accelerated by all the eight chemicals but only three of them increased the germination percentage significantly. The lecturer pointed out that after some modification the method might even have a practical application.

A. Pucarić (Zagreb, Yugoslavia) studied the correlation between leaf area per ha (called by him leaf area index) and yield as well as the net rate of assimilation in three hybrids of different vegetative periods, with five different growing spaces.

D. Palaversić (Zagreb, Yugoslavia) reported similarly on spacing experiments pointing out that the different response given by the hybrids to various population densities is able to change even the order of their yields. The influence of close spacing in decreasing the standability is not general, it is a function of variety and crop year. The next lecture was delivered by Kapás S. (Budapest) who on the basis of Hungarian variety trials spoke about the correlation of the amount of heat and precipitation to the yields of very early and medium late maize varieties.

The lecture delivered by T. Krivić (Ljubljana, Yugoslavia) on the excellent protein quality of flint lines selected from Slovenian local varieties and of their crosses should have been included in the first group discussing quality improvement.

Finally, A. Jakacki's (Kobierzyce, Poland) brief lecture described an interesting new method of using maize as fodder: producing meal by drying and grinding the whole plant (whole-corn meal). At last he summed up in seven points the advantages of this method intensely spreading since then.

The subjects of the sorghum group working as a separate group of the maize section were treated in three lectures. First R. Landi (Florence, Italy) spoke about the genetics and breeding of waxy sorghum containing starch in the form of amylopectine, presenting in both relations his own experiments too.

Then Fehér K. (Szeged, Hungary) gave detailed information on grain sorghum breeding at Szeged and on its results, including the economic aspects and the carotene contents of varieties and crosses too.

Finally, K. Sostarić-Pisačić (Zagreb, Yugoslavia) reported on the breeding of sweet sorghum used as green fodder in Yugoslavia, presenting the results of extensive variety trials too, in which, in addition to sweet sorghum varieties of various origin, varieties of other plant species competing with sweet sorghum were also included.

Apart from the lectures the book contains two reports on the work done by the two work-groups of the Section, and deals with matters of administrative character relative to the Section as well.

The volume thus gives a clear picture of the work of the Section, of the extent meetings held in every second year promote the progress of European maize breeding, offering the breeders a chance of getting acquainted mutually with the most recent results of their research.

L. Berzsenyi-Janosits

Búzatermesztési kísérletek 1960—70 (Wheat growing experiments 1960—70), (Editor: J. Bajai). Akadémiai Kiadó (Publishing House of the Hungarian Academy of Sciences), Budapest, 641.

The study collection "Wheat growing experiments 1960—70" published by the Publishing House of the Hungarian Academy of Sciences is the second one edited by Dr. J. Bajai. The first volume presented in 1961 showed the "yield" of eight years. The present book gives an account of eleven years' work.

Research results which for some reasons have not gone to press can be found in the desk drawers of most scientific workers. They often become lost without being useful either to research or to practice. Bajai's intention to try to save such studies is therefore highly justified.

The volume contains 60 papers apart from the meteorological summary. Most of them could have been published in any of the Hungarian or even foreign special periodicals or bulletins, still they have remained hidden. We should like to know why? We welcome the work, and hope that the useful practice which is beginning to become traditional will continue in the future too.

The papers are arranged in 9 chapters. One of the most difficult tasks for the editor was probably to classify the papers into groups. The agrotechnical research of wheat is a highly divergent subject, difficult to systemize by itself. It is not easy to find a method to set up categories which do not overlap each other. And in this case it is not even a matter of research work directed on the basis of the same principle, as the authors working at various institutes did not know when starting the experiments — perhaps not even when evaluating the results — that their papers would some day be included in a collection.

No judgement can be formed on the authors' choice of subject as a whole, since the papers represent only a part of their research work; they supposedly have reported in the meantime on other experiments of theirs in the different periodicals. Still, it cannot be left unmentioned that while the nature of some authors' subjects suggests that the authors wanted to satisfy immediate practical demands when choosing these subjects, in several cases the choice of subject was hardly influenced by practical considerations, and the authors started the experiments to have an opportunity to publish something.

As for the results, some of the older practising experts are of the opinion that theses known for decades in practice and taught as fundamental knowledge at the universities are repeatedly confirmed by experiments in the agrotechnical research of today. In certain cases research work of this type can be excused, because agriculture — including soil cultivation, fertilization, chemization, plant management and harvesting — has undergone a considerable transformation during the last 25 years, and it is not for nothing that earlier empirical findings are checked experimentally. Under the new conditions one or the other may need revision.

The editor could not influence the subjects, since he could only use what had remained hidden in the researchers' rooms, or was about to be completed. But if he could select, then it was his merit that the overwhelming majority of the papers contained interesting and useful data. They are not only very instructive but also give a cross-section of the problems that are today in the centre of interest. Unfortunately, the number of the papers does not reflect the importance of the subject, on the contrary, the papers dealing with better known subjects are represented in greater numbers. There sometimes occur "hearded" subjects too which are neither timely nor interesting. They generally give results which change according to variety, year, soil type and many other factors involved. The correlations cannot be proved even with the most up-to-date statistical methods, not to mention a number of factors which are not known in advance and could not even be influenced. These subject ought to be dropped at last, and the efforts concentrated to fields where immediate and essential results can be expected. We think that a part of the experiments on the time, depth and rate of sowing, and in some respects those on the rate, time and method of fertilization can be included in this category in the future.

The size of most papers is moderate. This is a very useful practice which suggests that not only the readers but the authors too have realized that the value of the papers is not always in positive correlation with the number of the pages. It is a pity that some authors have not yet adopted this useful practice, and try to raise the level of their studies with extensive tables, columns of figures and masses of diagrams, albeit they are today mostly omitted as nobody pays much attention to them. The presentation of results obtained by up-to-date statistical methods of evaluation as well as the degree of their reliability satisfy everybody. The summaries are mostly clear, easy to understand, and do not leave any doubt whether — or not — useful results have been obtained.

A welcome improvement compared to the previous volume is the introduction of foreign language summaries. This ensures a much wider publicity to the papers. Our requirements would be even more fully satisfied if the foreign summaries had at least the same extension as the Hungarian ones, or gave still more than that. In general, everybody reads the summary of a paper first and goes through the article circumstantially only if the summary has aroused his interest. Foreign readers are greatly assisted by detailed summaries as they need not translate the whole article. In a well constructed summary all that may be of interest for an expert can be told of a paper.

It is a regrettable deficiency of the volume — as compared to the previous one — that it contains very few pictures, and even those are of poor quality. True, good black-and-white photos are not easy to make and print of agrotechnical experiments on wheat, but photo-technics have improved for the last ten years, and typography ought to have improved as well. Unfortunately just the opposite can be seen. Some pictures in the book are of very bad quality. The 1961 edition contained much more and better photos. We hope this does not mean that the research workers make less photos. No research work can be made without photo documentation.

The way of presentation varies, naturally, with the authors, yet it can be felt that the editor has done a good work in this respect too.

The meteorological summary — found in the previous volume too — gives useful data.

Chapter II contains the highest number of papers. Biology and ecology seem to be preferred subjects. It is a pity that only two papers deal with the questions of wheat monoculture. In our opinion this subject is worth being intensely studied.

Chapter III which deals with soil cultivation contains only three papers. The question can be raised whether everything is so clear about soil cultivation? Namely, this is the very field in which mechanization has brought about the greatest changes. We do hope that there are extensive investigations in process which will increase the value of a third volume. Practice has shown, namely, that soil cultivation produces the most trouble.

Chapter IV discusses the problems of chemization. It appears that the foliage spray experiments carried out by Koltai and Pekáry have still not settled the question, although this subject too belongs to the older ones.

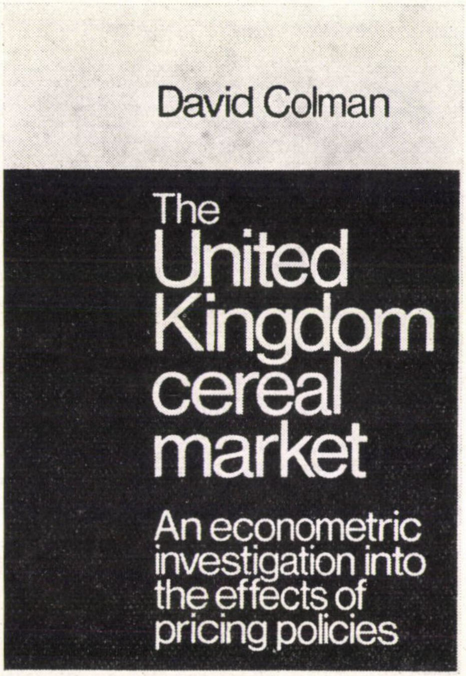
The papers of Chapter V deal with the subject of sowing. It is a deficiency that only two of the papers — small in numbers anyway — discuss the question of direct sowing, although it is a really new and economically important subject.

Chapter VI which deals with the problems of irrigation similarly only contains three papers. It is highly probable that this will be the most frequented field of research in the future.

The correlations of quality and various ecological factors are discussed with full particulars in three interesting papers written by Mrs. E. Pollhamer. It is a pity that the biological value of wheat protein, the question of lysine is dealt with only by a single group of authors.

The study collection "Wheat growing experiments 1960—70" is a worthy continuation of the first volume. It provides a series of valuable research results for the experts. It is a recent document of agricultural research work in Hungary, for which we are equally indebted to the authors and the editor. The Akadémiai Kiadó (Publishing House of the Hungarian Academy of Sciences) took pains over the artistic execution.

J. LELLEY



COLMAN, D.: *The United Kingdom Cereal Market*. An Econometric Investigation into the Effects of Pricing Policies. Manchester University Press, Manchester, 1972.

In the field of agricultural economics econometric analyses, aimed at disclosing technical and economic relationships, are often encountered. Econometrics is a means especially preferred in investigations aimed at acquiring a better knowledge of the efficiency of past or present economic policies with the view of elaborating more suitable ones.

The initiators and performers of such investigations as well as those utilizing the results must, of course, be aware of the fact that the efficiency of the methods depends, even within their own limits, to a great extent on the proper formulation and quantification of technico-economic relationships determining the phenomenon to be studied.

The author paid great attention to this

requirement while he prepared an investigation of two important means of economic policy: the deficiency payment scheme and the system of minimum import prices in the cereal branch of British agriculture. This required an analysis of the supply and demand of cereals and of the livestock trends.

I

A survey of economic policies on the basis of which the results and the conclusions drawn from them could be evaluated should be found — according to the table of contents — in the first two chapters of the book. Unfortunately, this important part is missing from the copy sent to our editorial office (while pages 17—32 appear twice).

Chapter III surveys the decision making conditions of the British farms and the major factors influencing the decisions. It is stated that natural conditions, through the rigidities of the production structure adjusted to them, cause a certain degree of inflexibility. The author points to the spatial location of the processing industries as one of the major determinants. He mentions the sugar industry as an example: 15 of the 17 English sugar factories are concentrated on the area of two eastern shires of England.

The volume of fixed assets invested is also a factor causing inflexibility. Its high purchase and very low salvage value, expenses of removal even possibly exceeding the latter, the rather limited possibility of alternative utilization inhibit the producer in his reacting flexibly to either declining or rising prices. The author points out that the rapid technical development shifts the supply function to the right. It has a decisive role from the point of view of estimating the functions of behaviour, since technical progress may strengthen the irreversibility caused by the fixed assets and, in the case of sufficiently intensive and rapid innovations, may offset or even exceed the price effect.

Apart from the various conditions (dimensions, specialization, relative profitability and capacity requirements, etc.), decision making, and supply too, are considerably influenced by the weather in certain phases of the period of vegetation.

The author stresses that a supply analyst must regard the decisions made by a great number of producers as a process, in its distribution over time.

The demand for cereals is analysed by its composition: as a demand for feed (purchased feed and even within this feed mixes dealt with separately), as human consumption and industrial utilization respectively, and as seed grain requirement. In this chapter fodder demand is discussed in great detail, with changes in feedstuffs, in feeding methods and requirements per unit of output being taken in consideration.

Data available did not allow to assess the total feed demand by demand functions specified for each kind of animal, so the aggregation method suggested by the author was not employed.

The author emphasizes the role of technical progress as a factor influencing the cereal demand serving the purpose of human consumption. It is manifest partly in varietal features (e.g. new varieties easier to grind are introduced in the production), partly in the technology of food industry (e.g. a new method of kneading made it possible for the baking industry to increase the proportion of soft wheat in the amount of flour used).

II

The dependent variable represented the harvested area, while as independent variables the following were taken into account: the price per unit of the product, unit prices of competing products, own yield, yields of competing cereals, yields of other competing agricultural products, further the inputs. As regards the cereal prices it must be emphasized that they were *always* considered as prices received by the producer, that is, they include the subvention too.

Starting from the above general specification, the author arrived at simpler forms by concentrating yield and price in a single variable as return from sales per unit area. On account of the information influencing the producer's decision, some of the variables are lagged, such as the acreage and the return per acre in the previous year, which may be accompanied by climatic variables relative to the current or previous year. Trend variables are often found in linear or quadratic or logarithmic form. The return per acre, lagged by one year, appears in a form of comparison too: either as difference between returns per acre of the studied crop and some competing product or as a ratio of them.

In the case of winter wheat, e.g., the following combinations of variables were used for purposes of estimation:

a) return per acre of winter wheat, return per acre of barley, meteorological indices for September, October and November (each of them lagged by one year) and the trend;

b) meteorological index for autumn (lagged) and trend.

In the case of summer wheat:

a) acreage of winter wheat in the current year, the meteorological indices for February, March and April and as lagged variables, the returns per acre of wheat and barley;

b) acreage of winter wheat in the current year, the ratio between the returns per acre of wheat and barley as a lagged variable, and the meteorological index for spring;

c) acreage of winter wheat in the current year, the trend and the lagged spring weather index.

Of the functions specified for barley:

a) the acreage of wheat in the current year, the trend, lagged variables are the acreage of barley and potato, the returns per acre of barley, oats, other cereals and from milk production;

b) acreage of wheat in the current year, as lagged variables the acreage of barley, the ratio of returns per acre of barley and oats;

c) acreage of wheat in the current year, the area producing rough fodder, with a lag the acreage of barley and potatoes, further the ratio between returns per acre of barley and oats.

In the estimation of the yields, the author started with the assumption that weather conditions in the critical phases of the vegetative period, fertilizer used in the previous crop year and the acreage belong to the most important explanatory variables. Weather was quantified by De Martonne indices for three months. Trend variables were also taken in consideration in linear, quadratic and logarithmic forms. In the case of wheat, the share of winter wheat from the total area under wheat was also used as an independent variable.

III

A whole series of demand functions were computed by the author for feed, human consumption and seed grains. Within feed he treated the individual cereals and the groups of high energy and protein feeds separately. Functions of demand for imported cereals could not be quantified (due to lack of adequate data), nevertheless with the aid of demand and supply functions and factual data on net imports, inferences concerning the cereal import can be drawn for periods on which stock data are available, and changes in the stock can thus be taken into account. Factors influencing the demand for feed included the livestock (expressed in animal units weighed by the feed consumption), the market prices of the kind of feed in question and of the other feed grains, the prices — with government subsidies included — of animals and animal products, the price of protein feed and the amount of the roughage fed.

Among the explanatory variables of demand for human consumption and industrial utilization we find the prices of the product (of adequate quality) in question and of the final product made from it, the number of population, the personal disposable income and the prices of competing products.

Demand for seed grains was quantified as a function of the acreage and of a trend variable.

To estimate the trends of livestock numbers, yearly and quarterly functions were used in various forms. Previous year's stock was used as an explanatory variable, prices of high energy and protein feeds similarly lagged, further the returns of the other groups of animals. To estimate the number of fattening animals the breeding stock was also taken into account as an independent variable.

IV

In order to test the functions for reliability of estimation, data of the period 1963/64 through 1970/71 were substituted into the functions relative to acreage and yields, while the evaluation of demand functions occurred by using data for the period between 1963/64 and 1967/68. The author considered the errors in estimates for wheat to be large as they exceeded several times 10 per cent, while in the case of the other cereals the error remained below 5 per cent almost every year. It was remarkable that the acreage of barley, oats and mixed cereals estimated by means of these functions even followed the sudden changes that occurred at the end of the period studied (decrease in the area of barley, increase in that of oats and mixed cereals), which was a further evidence of the reliability of these functions.

As for wheat, it must be stressed that, due to the decisive role of weather in September-October-November, projections for several years ahead should necessarily assume "normal" weather conditions, in the awareness of large potential errors in the estimates. It is an important statement, on the other hand, that on the basis of data on the December acreage of winter wheat, the area to be sown with spring wheat in the next year as well as the total harvestable wheat area can be estimated rather well even 9 months before the publication of the official statistical data.

It follows from the above that the estimation of volumes of output as the product of estimated yields and acreages was not so reliable in the case of wheat, while with the other cereals this procedure met the requirements better. It must be noted that the error criticized in the case of wheat exceeded 10 per cent in 3 of the 8 years, and in 3 years was close to it. In the case of mixed cereals there was only one year when the error of estimation exceeded 5 per cent.

Error of estimation of demand functions proved to be much smaller: it often did not even reach 1 per cent. It was surprising, on the other hand, that in a five year period certain functions with relatively lower coefficients of determination gave estimates closer to the factual data than the ones in which this character was substantially better.

An analysis of the price effects revealed that the acreage of winter wheat was not too responsive to the changes in the price of wheat, while the area under barley responded quite well (and positively !) to the changes in the wheat price. The joint increase of the guaranteed prices of wheat and barley would thus result in an increase in the barley acreage and hardly any change in that of wheat. This cross-effect warns the decision makers to choose the measures in accordance with the basic goal since the policy considered has more than one element.

Grain and milk production were shown as competing branches by the functions too: the price elasticity of the cereal acreage only slightly exceeded the absolute value of cross elasticity relative to the milk price.

The policy of minimum import prices would be aimed at reducing the subventions to the cereal producers on one hand, and the expenses in foreign exchange on the other. However, the author has drawn the conclusion from a deeper analysis of the system of functions discussed above that an increase in the minimum export prices would not lead to savings in foreign exchange even if the foreign suppliers did not increase the prices. In connection with this, the author refers to the fact that the effects of economic policy measures are much more complex than supposed by the officials sticking to surveys with traditional means. Indirect, not conspicuous but intense effects may thus be left out of consideration, although more consistent and thus more efficient economic policy measures could be taken on the basis of studies performed in time and with adequate methods.

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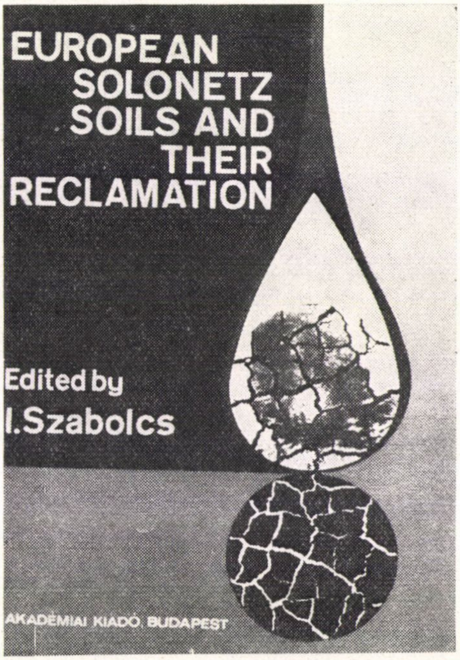

EUROPEAN
SOLONETZ
SOILS AND
THEIR
RECLAMATION

Edited by
I.Szabolcs

AKADÉMIAI KIADÓ, BUDAPEST

European solonetz soils and their reclamation (Ed.: I. Szabolcs), Akadémiai Kiadó, Budapest, 1971. 204.

The international fame of soil science in Hungary rests upon the results attained by Péter Treitz and Elek 'Sigmond at the beginning of the century. The present work which contains papers by Hungarian and foreign authors has been published by the Publishing House of the Hungarian Academy of Sciences as a continuation of this tradition.

István Szabolcs's review on the formation and characteristics of the alkali soils of Europe is followed by seven papers the authors of which give an account of the results attained in soil genesis and reclamation in relation with alkali soils.

Rajkov, L. offers a brief survey of the formation and reclamation of alkali soils in Bulgaria. He gives an account of the results of soil reclamation experiments carried out in the neighbourhood of Plovdiv, where beside a chemical amelioration deep ploughing and subsoiling were also applied. The experiments performed offer a possibility of successfully reclaiming alkali soil spots on a relatively small area.

Hrasko, J. reviews the alkali soils of Czechoslovakia and discusses the possibilities of making use of these soils. The process of alkalization in the three main alkali regions of Czechoslovakia — South-Moravia, the Danube-valley and East-Slovakia — is described with special attention to the role of the geological and geomorphological conditions. On the basis of a large number of data collected, in the course of a detailed soil mapping, valuable correlations are discussed between the trends of the various soil forming factors and the occurrence of alkalization. No report is presented either on reclamation experiments or on operative melioration, therefore this paper is one-sided compared to the others.

Ábrahám, L. and Bocskai, J. give account of the results of alkali soil research in Hungary in the largest paper of the book. They discuss the natural causes of alkalization and the effects of flood protection in detail. Profiles of major alkali soil types are described, and comprehensive limit values of investigation data on the various types are presented. Results of fertilization experiments, efficiency of chemical and physical soil amelioration are reported. After a brief survey of the history of alkali soil reclamation — which is, however, sufficient to give the reader a true picture of the results of this branch of science — demonstrative data of amelioration experiments and results obtained with afforestation, rice production and irrigation on these soils are presented.

Obrejanu, G. and Sandu, G. deal with the alkali soils of Roumania and sum up the results attained in their reclamation. Among the chemicals the effect of phospho-gypsum is described, while in the field of physical amelioration numerous data are presented on the results of salt leaching. On the basis of detailed investigations an account is given of the influence of amelioration methods on the distribution of exchangeable sodium within the soil profile as well as on the yield.

Grande Covián, R. presents the soil amelioration problems of Spain by the example of the saline soils in the delta of the Guadalquivir. These "marsh" soils contain a considerable amount of chlorides a high proportion of which is common salt. The preconditions of amelioration are here: release from the effect of sea-water, leaching with fresh water and improvement of permeability in the deeper soil layers. The author presents numerous data on the promising results, and utilization of these areas for livestock breeding is expected accordingly.

Pak, K. P. discusses the classification of alkali soils found in the European part of the Soviet Union and gives an account of the results attained with gypsum. Data of long-time experiments prove the favourable effect of chemical amelioration on both the chemical characteristics of soils and the crop yields attained on them.

Miljkovic, N. and Plamenac, N. describe alkali soils in Yugoslavia, in the regions of Vojvodina, Bácska, Bánát, Macedonia and Syrmia. Formation of these soils is discussed and their properties described on the basis of detailed geological and geomorphological explanations. The high boron contents of alkali soils which may be harmful in many cases, as well as the distribution of clay minerals in the different soil horizons are emphasized. On the basis of the latter data the alkalization theory of Gedroiz and 'Sigmond seems to be confirmed. In the soil reclamation section treatments with powdered sulphur may be outlined as resulting in favourable changes.

The above papers are followed by a bibliography compiled by György Várallyay, which is divided into two parts. In the first part titles of papers on the European alkali soils published between 1960 and 1969 are contained, while in the second part titles of bibliographies covering papers published before 1960 are presented. The two parts give full information on the literary sources thus offering a substantial help to experts dealing with or interested in the subject.

From the short enumeration we can see that the collection of papers is suitable to increase the international reputation of Hungarian soil science. By its comprehensive character it may have a mediatory role towards the soil researchers of other continents. The introductory paper of Szabolcs I. gives an appropriate frame to the papers and the bibliography compiled by Várallyay Gy. increases the completeness and use of the work.

On the basis of all this the publication of the book is welcome. Nevertheless there are some minor faults without which the work would still more valuable. This remark applies to some faults of construction and printing which, unfortunately, reduce the value of the book. First we have to mention the English language which varies from paper to paper to a considerable extent. The use of terms like "gypsuming" is not too felicitous. It is illogical that in many places in the text and tables the short form of milligram-equivalent is "me." while in the figures "meq.". Mistakes like "sojonetz" on the alkali soil map of Europe, CO_8^{2-} instead of CO_3^{2-} in the table on page 57, "T-érték" instead of "CEC" in the table on page 69 are conspicuous. The first line of page 99 is nonsensical, in the figure on page 103 NCO_3^- is found instead of HCO_3^-.

In spite of these technical deficiencies the book has reacted its purpose: it summarizes our knowledge of the alkali soils and helps in making the international value of the traditions and recent results of Hungarian alkali soil research known.

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РЕЗЮМЕ

ВЛИЯНИЕ 2,4-D НА АКТИВНОСТЬ ПОЛИФЕНОЛОКИСЛЕНИЯ ИЗОЛИРОВАННЫХ ТКАНЕЙ КАРТОФЕЛЯ

Е. КОВАЧ, Б. ФАЛУДИ

Изучалось влияние 2,4-D на активность полифенолокисления изолированных тканей картофеля. Прибавленный к препарату энзимов 2,4-D в концентрациях 10^{-7}—10^{-3}M не оказал влияния на активность энзимов. В культурах картофельных тканей, разведенных на питательной среде с различной концентрацией 2,4-D активность полифенолокисления оказалась различной. 2,4-D при концентрации 10^{-4}M слегка возбуждает активность энзимов в культурах тканей тогда, как при концентрации 10^{-3}M значительно задерживает активность фенолазы. Субстраты фенолазной системы в оболочке клубней картофеля следующие: кофейная, хлорогенная и изохлорогенная кислоты. И активность фенолазы, и содержание полифенола показали количественные изменения в зависимости от сорта картофеля.

ОПРЕДЕЛЕНИЕ ГУСТОТЫ, А ТАКЖЕ МАССЫ ПЫЛЬЦЕВЫХ ЗЕРЕН

ДЬ. ПАЛ, М. ПОКА

Густота пыльцевых зерен может быть определена методом гидростатической флотации, используя две свободно смешивающиеся жидкости, которые не растворяют, а только смачивают пыльцевые зерна. Жидкая смесь, в которой пыльцевые зерна плавают, везде может быть приготовлена из воды и глицерина. Флотация пыльцевых зерен, то есть гидростатическое равновесие наступает тогда, когда густота у жидкой смеси станет той же самой, как у пыльцевых зерен. Поэтому определение густоты пыльцевых зерен можно свести к простому определению густоты жидкости, что можно легко и быстро сделать с помощью шкалы **Mohr-Westphal**. У поры пыльцевых зерен плазмалемма не повреждается жидкостью, используемой для измерения потому, что они тесно связаны. Воздушные пузыри, прикрепленные к поверхности пыльцевых зерен, а также поверхностная неровность (утолщение и аппозиция) сексины устранимы употреблением очистительных средств. Если пыльцевые зерна оставляют в измерительной жидкости в течение длительного периода, появляется седиментация, в результате увеличивающейся густоты за счет обезвоживания цитоплазмы.

ИССЛЕДОВАНИЕ ФИТОМАССЫ НА СОЛОНЦОВОМ ПАСТБИЩЕ
(Achilleo-Festucetum pseudovinae) II

И. МАТЕ, И. ПРЕЧЕНИ

Исследования по количеству фитомассы проводились с 1969 по 1971 г. на солонцовом пастбище (Achilleo-Festucetum pseudovinae; Уйсентмаргита, показательная территория IBP), а также на поле, выключенном из пастьбы. Вес частей над уровнем почвы определялся по образцам скашивания, а вес частей под уровнем почвы по монолитным образцам, после высушивания при 105°C. Авторы изучают связь между климатическими условиями и продуктивностью; количеством продукции, числом зерен в почве. Рассматривается продуктивность, время смены веществ и темп образования продукции у ***Festuca pseudovina***, а также у частей над и под уровнем почвы.

НЕСКОЛЬКО ЦИТОЛОГИЧЕСКИХ И МОРФОЛОГИЧЕСКИХ ПРИЗНАКОВ У ОКТОПЛОИДНОГО ПОСЕВНОГО ЭСПАРЦЕТА *(Onobrychis viciifolia Scop.)*, ИНДУЦИРОВАННОГО КОЛХИЦИНОМ

И. БОЧА, Р. КИШКЕРИ, Ш. ХЕЙЙА

Из сортов автотетраплоидного посевного эспарцета ($2n = 4x = 28$) Матра и Комполти, а также из их поликроссного потомства путем обработки колхицином получился октоплоидный посевной эспарцет ($2n = 8x = 56$). Изучались главные цитологические и морфологические признаки двух уровней плоидии. Отношение экземпляров с 8n оказалось сравнительно постоянным (86—95%) в генерациях C_1—C_5, в то же время найдены многочисленные анеуплоидные формы, частота которых менялась в некоторых генерациях, но оказалась сравнительно большой. Степень обратной регуляции на тетраплоидный уровень, и также отношение гексаплоидов ничтожны, и ими можно пренебречь. С точки зрения изученных цитологических признаков — как размер клетки и устьица, хлоропластов в устьицах и пыльцевых зерен — особи с 8n превышают формы с 4n. Изученные формы с 8n превышают особи с 4n и по ряду морфологических признаков листьев и частей цветка, так напр. больше по размеру семядольные листья, простые и сложные листья, больше вес листочек и листьев, площадь листочек, черенок более толстый. По размерам частей цветка октоплоидный посевной эспарцет тоже превышает особи с 4n (парус, лодочка, крылья и «половой столб»). В фенофазах наблюдается смещение, т. е. рост и развитие форм с 8n более затянуты.

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EFFECT OF 2,4-D ON THE POLYPHENOLOXIDASE ACTIVITY OF ISOLATED POTATO TISSUES

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The effect of 2,4-D was studied on the polyphenoloxidase activity of isolated potato tissue. 10^{-7}—10^{-3} M concentration of 2,4-D added to the enzyme preparation had no influence on the enzyme activity. In potato tissue cultures grown on culture media with various 2,4-D concentrations the activity of polyphenoloxidase is different. A 2,4-D concentration of 10^{-4} M slightly stimulates the enzyme activity in the tissue cultures, while that of 10^{-3} M inhibits the phenolase activity to a great extent. The substrates of the phenolase system in the skin of potato tubers are: caffeic acid, chlorogenic acid and isochlorogenic acid. Both phenolase activity and polyphenol content showed quantitative differences depending on the potato varieties.

Introduction

During our experiments studying the effect of 2,4-D on the growth and metabolism of potato tissue cultures, we found that the degree of browning was dependent on the concentration of 2,4-D. Lower concentrations of 2,4-D caused a darker brown colour of the tissues while in the presence of higher 2,4-D concentrations the explants remained lighter. The phenomenon suggested that the 2,4-D may have influenced the activity of the phenoloxidases.

In the browning processes of plant tissues and organs researchers have long attached a great importance to polyphenoloxidase, tyrosinase. The processes of browning have been thoroughly investigated in tea and tobacco leaves, potato tuber and egg-fruit (Hattori—Shiroya 1955, Zucker—Stinson 1960, Knapp 1961). Some authors pointed out that tissue browning could be inhibited with substances containing —SH (e.g. cysteine, glutathione, Henze 1956) and with other reducing substances (e.g. ascorbic acid, Dawson—Magee 1955, Makower 1964).

In our experiments we set the aim of studying more closely the effect of 2,4-D on browning in potato tissues and the activity of the phenoloxidases.

Material and Method

The experiments were performed with the varieties "Gülbaba" and "Margit" of *Solanum tuberosum.* The experimental material was obtained from the National Research Institute of Agrobotany and also grown at the Biological Station of the Eötvös Loránd University at Alsógöd.

From the internal flesh of potato tubers (variety "Gülbaba") slices of 5×5 mm and 25—26 mg average weight were cut and placed under sterile conditions on an agar culture of a modified White medium. In addition to the mineral substances the culture medium contained the following compounds: 0.1 mg/lit aneurine, 0.5 mg/lit pyridoxine, 0.5 mg/lit nicotinic acid, 2.0 mg/lit glycine, 75 mg/lit enzymatic hydrolysed caseine (Amparon), 2 per cent saccharose, 1 per cent agar. 2.4-D was used at concentrations of 10^{-7}, 10^{-5}, 10^{-4} and 10^{-3} M, while variants without 2,4-D were used for control. The cultures were kept in the dark, at a temperature of 26 °C. Samples were taken on the 5th, 10th and 15th day.

Explants freed from the agar and weighed were frozen and an acetone-dry powder was prepared from them (Dawson—Magee 1955). The acetone-dry powder was extracted with a 0.15 M phosphate buffer (2 ml/g dry powder, pH = 6.8) over 12 hours at 1—3 °C. The extract was filtered and this filtrate, used as enzyme containing preparation.

The polyphenoloxidase activity was determined on the basis of oxygen decrease by Warburg's manometric method. The reaction mixture contained 0.2 ml enzyme preparation, 1.3 ml 0.15 M phosphate buffer (pH = 6.8) and 0.5 ml 0.056 M chlorogenic acid solution. (The final volume was 2.0 ml.) Activity was measured at 25 °C. Oxygen decrease was checked every two minutes.

The nitrogen content of the preparation was determined, after sulphuric acid destruction (Kjeldahl), from the extinction value obtained by the photometry of the colour produced with Nessler's reagent. Enzyme activities were expressed in μ lit. O_2 decrease per μg N per hour.

The extraction of polyphenols was carried out on the basis of data given by Johnson—Schaal (1952) after some modifications. The weighed skin of tubers was homogenized with sea sand with 96 per cent ethanol, and sodium bisulphite (0.3 ml 10 per cent bisulphite/g fresh weight) was used to inhibit the phenolases. Extraction was performed with 96 per cent ethanol twenty times the amount of the fresh weight at 55 °C for 2 hours. The extract was filtered, dried, then the dry residue was dissolved with 96 per cent ethanol at a ratio of 0.5 ml/g fresh weight. Chromatography was performed on Schleicher-Schüll 2043/b paper in one dimension. The chromatograms were developed in a mixture of butanol : acetic acid : water of a ratio of 4 : 1 : 5, or in 10 per cent acetic acid (Block *et al.* 1958) over 15 hours. Fluorescence of spots was studied. Chromatograms neutralized in ammonia gas were developed with a 0.1 per cent solution of $FeCl_3$, diazotated p-nitranilin plus 20 per cent Na_2CO_3 (Block *et al.* 1958).

For quantitative determination an amount of extract corresponding to 2 g fresh weight was chromatographed. The spots were cut out and the polyphenols eluated with 6 ml distilled water. The eluate was examined in an UVIFOT photometer at a wave-length of 313 millimicron. (Although the absorption maximum of the chlorogenic acid is 326 millimicron, on the basis of comparative determinations made with Beckman's spectrophotometer we found that measuring at a wave-length of 313 millimicron did not affect the correctness of our results. (The extinction values were expressed in chlorogenic acid quantities on the basis of a standard curve made with chlorogenic acid.)

The results of the experiments were obtained from three parallel measurements of four experiment series.

Results

The first step was to find out how the various concentrations of 2,4-D influenced in an in vitro enzyme preparation the activity of the polyphenolase enzyme. The results of the experiments are shown in Table 1.

From Table 1 it can be seen that the polyphenoloxidase activity is identical at all the applied 2,4-D concentrations, that is 2,4-D does not directly influence the activity of the polyphenoloxidase enzyme.

The following step was to study the polyphenoloxidase activity in explants of potato tuber tissues grown on culture media of different 2,4-D concentrations.

Table 2 shows the polyphenoloxidase activity of 5, 10 and 15 days old tissue explants.

Table 1

Effect of various concentrations of 2,4-D on the polyphenoloxidase activity of in vitro enzyme preparations (expressed in μlit. $O_2/\mu g$ N/hour)

Time of determinations	Untreated	2,4—D concentrations			
		10^{-7} M	10^{-5} M	10^{-4} M	10^{-3} M
2 minutes	106	110	108	103	105
4 minutes	212	221	217	208	212
6 minutes	327	329	322	312	327
8 minutes	431	438	429	415	430

Table 2

Effect of various 2,4-D concentrations on polyphenoloxidase activity in 5, 10 and 15 days old tissue cultures (μ lit. $O_2/\mu g$ N/hour)

Age of tissues	Time of determinations	Untreated	2,4-D concentrations			
			10^{-7} M	10^{-5} M	10^{-4} M	10^{-3} M
5 days	2 minutes	97	99	77	73	44
	4 minutes	195	199	158	146	88
	6 minutes	288	292	232	214	137
	8 minutes	378	376	305	272	187
10 days	2 minutes	132	119	104	161	27
	4 minutes	259	243	208	308	60
	6 minutes	382	353	299	470	82
	8 minutes	494	458	384	617	104
15 days	2 minutes	202	213	201	281	38
	4 minutes	400	403	388	562	76
	6 minutes	608	611	562	796	105
	8 minutes	806	780	736	1007	133

In 5 days old tissues the enzyme activity of the 2,4-D-free control and that of explants grown on culture media containing 2,4-D at a concentration of 10^{-7}M can be considered identical. On the other hand, in the presence of 2,4-D at concentrations of 10^{-5} and 10^{-4}M the polyphenolase activity slightly decreases. The decrease of enzyme activity is the highest in the case of a 2,4-D concentration of 10^{-3} M. In this case the tissues do not turn brown, while in the case of the control and lower concentrations of 2,4-D a very slight browning can be observed.

In the 10 days old tissues the changes are even more conspicuous. As compared to the untreated control the 2,4-D at a concentration of 10^{-7}M had an insignificant effect on the polyphenoloxidase activity, while at 10^{-5}M inhibited it to 22 per cent. It is interesting that at a 10^{-4}M concentration 2,4-D increases enzyme activity by 24 per cent. On the other hand, at a 10^{-3}M 2,4-D concentration a 79 per cent inhibition can be observed.

Untreated control tissues have the darkest brown colour, tissues grown on culture media with 2,4-D concentrations of 10^{-7}M and 10^{-5}M become less brown, while in the presence of 2,4-D at a concentration of 10^{-4}M — when growth is optimum — and 10^{-3}M the tissues do not turn brown.

On the 15th day differences in enzyme activity become still more pronounced (Table 2). The table shows that 10^{-7} and 10^{-5}M concentrations of 2,4-D hardly influence the polyphenolase activity of tissues compared to the control (2 and 9 per cent respectively). The growth stimulating concentration of 10^{-4}M enhances the oxygen decrease here, too (24 per cent). The extent of O_2 consumption corresponds to the change of activity found in the 10 days old tissues. In the case of the growth inhibiting 10^{-3}M concentration of 2,4-D the inhibition of enzyme activity is of extremely high extent (83 per cent). Browning shows a tendency similar to that found in the 10 days old tissues.

It can be seen from the above that in vivo 2,4-D influences the polyphenoloxidase activity of tissues indirectly. When comparing the enzyme activities of 5, 10 and 15 days old tissue cultures we find that with the exception of the 10^{-3}M concentration of 2,4-D the polyphenoloxidase activity increases in the 10 and 15 days old tissues compared to the 5 days old ones. In the case of the 10^{-3}M concentration, on the other hand, a decreasing tendency prevails. Further investigations are needed to find out which metabolic pathways are affected by the indirect effect of 2,4-D.

Subsequently we examined whether polyphenoloxidase activity was different in potato varieties giving genetically different responses to 2,4-D. Table 3 shows that the phenolase activity is not uniformly distributed in the potato tuber. Polyphenoloxidase activity is lower in the inner flesh of tubers than in the skin.

It is interesting that the polyphenoloxidase activity is identically high in the skin of the 2,4-D resistant "Margit" variety and the sensitive "Gülbaba" variety. In the flesh of the tubers the enzyme activity — while lower — showed decided differences between the sensitive and resistant varieties. In the resistant "Margit" variety the flesh of the tuber displayed a higher polyphenoloxidase activity than that of the sensitive variety. These results suggest that the differences of resistance to 2,4-D are shown in the differences of phenolase activity in the flesh of potato tubers, too.

Further we examined which natural substrates of the polyphenoloxidase enzyme occur in the tubers. In the inner flesh of tubers our investigations pointed out polyphenol derivatives only in traces. In the skin of tubers, on the other hand, polyphenols were found in considerable amounts. On the chromatograms of extracts obtained with ethanol five fluorescent spots were separated which on the basis of the reagents used could be considered polyphenol derivatives (Table 4).

Table 3

Changes in polyphenoloxidase activity in the tubers of various potato varieties ($\mu l\ O_2/\mu g\ N/hour$)

Time of determinations	Variety			
	Gülbaba		Margit	
	skin	flesh	skin	flesh
2 minutes	232	86	244	174
4 minutes	465	171	490	347
6 minutes	685	256	733	522
8 minutes	925	339	975	695

Of the fluorescent spots, spot "E" was identified as caffeic acid, spot "D" as chlorogenic acid on the basis of their location in the different solvent systems (R_f), chemical reactions and the parallel chromatography of standard chlorogenic acid and caffeic acid respectively. According to our investigations and on the basis of literary references (WEAWING 1958) spot "C" might be regarded as isochlorogenic acid. Spots "A" and "B" were not identified.

Of the polyphenol derivatives found on the chromatograms spots "C", "D" and "E" could also be determined quantitatively. It is caffeic acid that is generally found in the largest amounts, chlorogenic acid is less, and isochlorogenic acid occurs in a still lower quantity (Table 4). Spots "A" and "B" were

Table 4

R_f values and colour reactions in the chromatograms of polyphenol derivatives found in the skin of the tuber

Characteristics and reagents	Fluorescent spots				
	A	B	C	D	E
R_f (butanol, acetic acid, water = 4 : 1 : 5)	0.10	0.20	0.41	0.45	0.74
R_f(10% acetic acid)			0.65	0.68	0.39
UV fluorescence	blue	greenish	bluish-green	blue	blue
Ammonia gas	yellow	yellow	yellow	yellow	yellow
NH_3 + 0.1% $FeCl_3$	steel-grey	pale grey	greyish	greenish-grey	dark grey
Diazotated p-nitraniline	red	red	red	brownish	brown
Identified matter	?	?	(?) isochlorogenic acid	chlorogenic acid	caffeic acid

not quantitatively determined. On spot "B" the compound is generally present in the lowest quantity, the material content of spot "A" is somewhat higher though it is not significant compared to the amount of isochlorogenic acid. According to Table 5 the amount of polyphenols was higher in the variety "Margit" than in the "Gülbaba" at the time of the investigations.

The polyphenol content of the variety "Ella" is higher than that of the "Gülbaba" but lower than the polyphenol content of the variety "Margit."

Table 5

*Polyphenol content in the skin of tubers of various potato varieties (mg/g fresh weight)**

Polyphenols	Potato varieties		
	Gülbaba	Ella	Margit
Caffeic acid	0.250	0.875	1.125
Chlorogenic acid	0.250	0.425	0.875
Isochlorogenic acid(?)	0.125	0.250	0.250

* Values are expressed on the basis of the chlorogenic acid standard curve.

Discussion

Our experiments have revealed that in vivo 2,4-D influences the polyphenoloxidase activity of potato tissues. According to our experiments 2,4-D only increases the phenolase activity by 24 per cent at the growth stimulating concentration of 10^{-4}M. This concentration is higher than that used by others. MOREL—DÉMÉTRIÁDES (1955) observed the stimulation of polyphenoloxidase activity in artichoke tissue cultures when 2,4-D was applied at a concentration of 10^{-6}M. SPURR *et al.* (1962) cultured normal and crown-gall tissues of tomato on culture media with and without a 2,4-D content and studied the oxidizing activities of ascorbic acid and chlorogenic acid in the tissue homogenates. Normal tissues grew better on culture media containing 2,4-D than on those without a 2,4-D content, nevertheless the enzyme activities studied by the authors showed higher values in tissues grown on a 2,4-D-free culture medium than in those grown in the presence of 2,4-D. The growth of tissues isolated from a crown-gall was slower, and their enzyme activity lower in a culture medium containing 2,4-D in comparison with tissues grown on a 2,4-D-free culture medium.

The concentration of 2,4-D used by SPURR *et al.* (1962) corresponds to $2{,}71 \times 10^{-5}$M (6 mg/lit), i.e. it is lower than that found to be stimulatory in our experiments in normal tissues. On the other hand, the inhibition of phenolase activity occurred at a higher concentration of 2,4-D in our experiments. These differences may be due to the fact that the culture media the authors mentioned using were others than those used in our experiments and they added coconut milk to them which also contained factors affecting the relation between 2,4-D and the phenolase system. The diversity of the experimental objects may also have contributed to the differences.

2,4-D may exert its inhibiting effect on the phenolase activity by damaging the mitochondria as the polyphenoloxidase activity was localized in the mitochondria (McClendon 1953). There is evidence of 2,4-D influencing the mitochondrial functions: oxidative phosphorylation and other enzyme activities (Key *et al.* 1960, Wedding—Black 1962).

Since 2,4-D may cause the destruction of cell membranes (Faludi *et al.* 1959) it can be supposed that changes in the mitochondrial membranes involve changes in the function of the phenolase system. No close correlation was found between the browning and growth of tissues.

In our experiments 2,4-D was found to have no effect on the activity of in vitro enzyme extracts. This agrees with the results obtained by other authors, trying to influence other enzymes (Black—Humphreys 1962).

Further investigations may throw light upon the mechanism of the indirect effect of 2,4-D. In the potato varieties examined by us the substrates of the phenolase system are mainly caffeic acid and chlorogenic acid. These polyphenols are not uniformly distributed in the tuber: the skin of the tuber contains a considerably larger amount of polyphenol. This was observed by other authors too (Cheng—Hanning 1955, Crafts *et al.* 1958). The amount of chlorogenic acid and caffeic acid was found to vary in the different potato varieties. From this fact, however, no far-reaching conclusions can be drawn on the specific differences, as it has been pointed out by a number of research workers that the polyphenol content of potato tubers is highly influenced by the conditions of cultivation and storage as well as by climatic factors (Crafts *et al.* 1958, Wolfgang *et al.* 1959). The stimulation of the enzyme activity could be explained by gene activation.

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DETERMINATION OF THE DENSITY AND MASS OF POLLEN GRAINS

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The density of pollen grains can be determined by the hydrostatic floatation method in two freely intermixing liquids which do not dissolve only wet the pollen grains. A liquid mixture in which pollen grains remain floating everywhere can be prepared from water and glycerine. The floatation of pollen grains, that is the hydrostatic equilibrium sets in when the density of the liquid mixture is exactly the same as that of the pollen grains. The determination of pollen grain density can thus be reduced to a simple determination of liquid density which can be carried out easily and quickly with Mohr-Westphal scales. At the pores of the pollen grains the plasmalemma is not damaged by the measuring solution coming into close contact with it. Air-bubbles adhering to the surface of pollen grains and superficial unevenness (thickening and apposition) of the sexine can be removed by the application of detergents. In the case of pollen grains standing in the measuring solution for a longer period sedimentation can be observed as a result of the increasing density of the dehydrating cytoplasm.

Introduction

The size, first of all the diameter of pollen grains is known in most plant species (Erdtman 1954, 1965). We even know that the distribution of pollen grains according to diameter gives a one-peak curve in plants with monoecious and androgynous flowers and a typically two-peak curve in dioecious plants (Greguss 1927a, b). Consequently, pollen grains of dioecious plants show not only a genetical but a morphological (i.e. manifest in dimensions) dimorphism too. It has been established, further, that the male sex is determined by the larger while the female sex by the smaller pollen grains (Greguss 1929a, b). No literary data are, however, available — or even known — on the density and mass of pollen grains in the different plants.

Material and Method

The liquids used in our investigations were chosen taking various requirements in consideration: 1) they should freely mingle with water, have as high a density as possible compared to the water so as to make the preparation of liquid mixtures of any density possible; 2) they should not cause any change in the structure of the plasmalemma at the pores of the pollen grains (in this sense e.g. alcohol cannot be used); 3) pollen grains should be fully wetted in the liquid mixture by the use of a detergent.

Taking the above requirements in consideration the liquid mixture was made of water and glycerine (ϱ_{20} °C= 1, 2, .). According to our observations pollen grains do not readily become wet in clean water or in a mixture of water and glycerine. This fact must not be left unnoticed, because as a consequence of insufficient moistening tiny air-bubbles may adhere to the surface of pollen grains and get between the superficial unevenness (thickening and apposition) of the sexine thereby distorting the results of measuring to a great extent. Therefore to attain the necessary degree of moistening we applied Triton X-10, a non-ionic detergent at a very low concentration.

For a measuring instrument we used Mohr—Westphal scales which made it possible to determine the density very precisely and easily. The glass tube belonging to the instrument is at the same time a thermometer, so the temperature of the liquid mixture can be read simultaneously.

The method is based on a physical law (ERDEY-GRUZ—PROSZT 1950). According to the Archimedean principle a solid body floats (is in state of equilibrium) in a fluid when the resultant of the forces acting on it is zero, that is, when the lifting power of the fluid ($\vec{F}_f$ = density of fluid × volume of fluid × gravity acceleration) is equal to the weight of the solid body ($\vec{G}_{sz}$ = mass of solid body × gravity acceleration):

$$\vec{F}_f + \vec{G}_{sz} = 0, \text{ or } \vec{F}_f = -\vec{G}_{sz}$$

Accordingly we had to prepare a mixture of glycerine and water of a ratio that enabled the pollen grains to remain in a state of floatation for a considerable time, without any visible sedimentation or separation on the surface of the measuring fluid. In practice this is carried out in the following way: the two fluids are mixed at a more or less adequate ratio, the pollen grains are placed in the mixture and the direction of their movements followed. If a sedimentation is observed the density of the mixture is increased by adding glycerine until the floatation of pollen grains is attained; in the opposite case the density of the mixture is decreased by adding water. Light transmitted through the fluid mixture makes it much easier to observe the floatation of pollen grains. If in this way the condition of equilibrium has been created, that is the pollen grains are floating in the liquid mixture, then the density of the latter can be measured directly with Mohr—Westphal scales, and the value obtained is — according to the Archimedean principle — equal to the value of the pollen grain density. The density of the pollen grains was determined in the species and varieties: *Calystegia sepium* (L.) R. Br., *Petunia atkinsiana* Don., *Dahlia pinnata* Cav., *Verbascum phlomoides* L., *Gomphrena globosa* L., *Cleome spinosa* L., *Salvia splendens* Sellow, *Cucurbita pepo* var. *ovifera* L., *Hibiscus syriacus* L., *Zea mays* L.

The mass of the pollen grains can be obtained from their density multiplied by their volume ($\varrho = m/V$; $m = \varrho V$). The volume of pollen grains can be calculated if their diameter is determined ($V = 1/6\, \pi\, d^3$). If gravity acceleration in the case of pollen grains (g) is regarded as negligible, the values of density and specific weight can be considered identical ($y = m\, g/V = \varrho g$, as $m/V = \varrho$).

Results

Density and volume of pollen grains. The pollen grains of different plants are not of the same shape. There are isodiametrical and anisodiametrical pollen grains. In the case of anisodiametrical pollen grains the two diameters are not of identical size, that is, the lengths of the small and large axes are different. For pollen grains of this type the average of the two diameters was taken into account in our calculations. These pollen grains were corrected into spheres and their volume thus computed.

The density of the pollen grains studied by us ranged between 1.110 g/cm^3 and 1.248 g/cm^3 depending on the species (Table 1). At the same time air density varied from 0.001173 to 0.001197 g/cm^3. The diameters of the pollen grains ranged between 26.04 and 160.04 millimicron, and their volume was, accordingly, between 9.22×10^{-9} cm^3 and 2145.28×10^{-9} cm^3. The mass of the

Table 1

Characteristics of pollen grains

Plant species studied	Temperature of the mixture C°	Density of pollen grains g/cm³	Air density at the temperature of the mixture g/cm³	Pollen grain		
				diameter μm	volume $\times 10^{-9}$ cm³	mass $\times 10^{-9}$ (g)
Calystegia sepium (L.) R. Br.	28	1.110	0.001173	91.08	394.70	438.12
Petunia atkinsiana Don.	28	1.118	0.001173	33.48	19.53	21.83
Dahlia pinnata Cav.	27	1.128	0.001177	38.64	30.13	33.99
Verbascum phlomoides L.	26	1.137	0.001181	29.58	13.58	15.44
Gomphrena globosa L.	27	1.149	0.001177	36.06	24.43	28.07
Cleome spinosa L.	25	1.151	0.001185	26.04	9.22	10.61
Salvia splendens Sellow.	25	1.153	0.001185	64.26	138.61	159.72
Cucurbita pepo var. *ovifera* L.	25	1.168	0.001185	159.72	2137.32	2496.39
Hibiscus syriacus L.	22	1.232	0.001197	160.04	2145.28	2642.97
Zea mays L.	27	1.248	0.001177	84.78	319.40	398.61

pollen grains varied from 10.61×10^{-9} g to 2642.97×10^{-9} g. Thus the mass of a pollen grain is primarily determined by its diameter. Namely, the order of pollen grains determined by the diameter corresponds to the volumetrical order and is not affected by the density. The density of the pollen grains in the studied plant species is significantly higher than air density at a given temperature.

The behaviour of sterile and fertile pollen grains. Among the pollen grains there are sterile and fertile ones at a ratio depending on the species. When determining the density of pollen grains the fertile pollen grains were always taken as a basis. Namely, the sterile pollen grains are always found on the surface of the measuring solution, since their density is considerably lower than that of the fertile ones. The supernatant (pollen grains on the surface of the measuring solution) does not hinder the process of measuring, and can easily be removed.

Temporal changes in the density of pollen grains. The density of pollen grains was determined immediately after they had been collected. It was

Table 2

Temporal changes in the density of pollen grains

Species	Density of pollen grain			Changes in density	
	when collected	after 6 hours	after 18 hours	after 6 hours	after 18 hours
Verbascum phlomoides L.	1.137	1.150	1.162	0.013	0.012
Dahlia pinnata Cav.	1.128	1.160	1.167	0.032	0.007

a problem whether the value of density would change if the pollen grains were left in the measuring solution for different periods of time. Changes in the density of pollen grains kept for different times in the measuring solution are shown in Table 2. The density of the pollen grains from the two species examined increased parallel with the time they were kept in the measuring solution. The rate of increase in the density value was higher in the first six hours than in the subsequent 18 hours in both species. The differences in the increase of density between the dates of measuring thus decrease with the passing of time This means that the increase in the value of pollen grain density shows a decreasing tendency with advancing time.

Discussion

The determination of the density and mass of pollen grains makes it possible to acquire a more exact knowledge of the methods of pollination. The mass of the pollen grain is determined first of all by its diameter, so they increase their mass mainly by enlargening their volume, and only to a lesser degree by a greater density. This statement is confirmed by the fact that while the diameters of pollen grains studied by us ranged between 26.04 and 160.04 millimicrons, their density only between 1.110 and 1.248 g/cm^3. The described method of determination does not damage the plasmalemma coming into close contact with the measuring solution through the pores of the pollen grains, as it was not found to be disintegrating. While the pollen grains are standing in the measuring solution, a material transport takes place through the plasmalemma at the pores by an active or passive diffusion. Namely the pollen grains, while standing in the measuring solution, increase in density which means that matters contained in the pollen grains become concentrated, and this occurs only if the pollen grain loses water. The loss of water — plasmolysis — of the protoplasma involves a reduction of volume. The pollen grain itself cannot shrink, since it is protected by a non-shrink exine resistant to acids and alkali. Thus the actual volume of the pollen grain cannot be reduced. However, in the pores, by the cytoplasm trabeculae interweaving the intine the plasmalemma is in direct contact with the measuring solution, and plasmolysis may thus occur here. The plasmalemma coming into contact with the measuring solution plasmolyses at the pores and gets separated from the inner surface of the intine by which the actual volume of the cytoplasm decreases. The place of the contracting cytoplasm is occupied through the exine lattice of the pores by measuring solution, and through the plasmalemma by water evacuating the cytoplasm. Thus the volume of cytoplasm losing water will decrease while its concentration — and thereby its density too — increase. Within the pollen grain the measuring solution entering the space left blank by the contracting cytoplasm will be diluted owing to the water leaving the cytoplasm. If the joint density of the concentrating cytoplasm of the pollen grain, the measuring solution entering the pollen grain and the water leaving the cytoplasm is higher than the density of the measuring solution, sedimentation begins. This, however, only occurs after standing for a considerable time. Since the determination of floatation requires a very short time (5—10 minutes), while sedimentation begins only after several hours, this method of determination gives the real density of the collected pollen grains.

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SOME CYTOLOGICAL AND MORPHOLOGICAL PROPERTIES OF OCTOPLOID SAINFOIN (ONOBRYCHIS VICIIFOLIA SCOP.) INDUCED BY COLCHICINE

By

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By treating the autotetraploid sainfoin varieties Mátra and Kompolti (2n = = 4 × = 28) with colchicine we obtained octoploid sainfoin plants (2n = 8 × = 56). We studied the major cytological and morphological properties of the two different ploidy levels. The percentage of octoploid plants in the C_1 — C_5 generations was relatively constant (86—95%), at the same time numerous aneuploid forms were also found, the frequency of which — though varying in the individual generations — was relatively high. The extent of re-regulation to the tetraploid level as well as the percentage of hexaploids were negligible. As to the cytological characteristics examined — the cell and stoma size, the chloroplasts found in the guard cells of stomatal pores, the pollen size — the octoploid plants were superior to the tetraploid forms. The octoploid forms studied surpass the tetraploid plants in the morphological properties of the leaf- and flower parts too; so the cotyledons and the first simple and compound leaflets are larger, the leaf- and leaflet weight greater, the surface of the leaflet larger and the petiole thicker. The measurements of the flower parts (standard, keel, wings and staminal column) in the octoploid sainfoin exceed those of the tetraploid forms, too. There is a shift in the phenophases, namely the growth and development of the octoploid forms are protracted.

Introduction

Although the culture varieties of cultivated sainfoin (*Onobrychis viciifolia* Scop., *Onobrychis sativa* Lam.) in their relatively narrow zone of cultivation generally meet the requirements expected of them, it is a well-known fact that the stalks soon become lignified, are inclined to lodging, and the ratio of leaf to stalk is unfavourable. The available ecotypes and culture varieties do not represent a higher genetic variability in this respect either, so the possibility of selection is limited.

To increase the variability of the initial material the method of polyploid breeding was chosen, in spite of the fact that the autotetraploid character of the varieties belonging to the same morphological group as the *Onobrychis viciifolia* had been known for nearly forty years. Thus according to Darlington—Wylie (1955) Romanenko in 1939 and Mande in 1939 described a chromosome number of $2n = 4x = 28$ for *Onobrychis viciifolia.* Similar data were published by Tischler (1950) and Badoux (1964). (The latter also listed the diploid species.) Under such conditions the aim was to produce octoploid forms, the $2n = 8x = 56$ chromosome number of which may appear to be beyond optimum. To our knowledge such forms have not been developed so far.

The present paper describes some of the cytological and morphological properties of these octoploid forms.

Material and Method

The state registered varieties Kompolti and Mátra and their polycross progenies were used in the colchicine treatments.

The treatment was applied to 200 plants of each variety. During the period of the treatment the germinated seeds were kept in a glasshouse of 18—20 °C, protected against sunshine. The first treatment was performed by dropping a 1 per cent colchicine solution between the two cotyledons. After two days the treatment was continued with a 0.1 per cent colchicine solution, and each subsequent treatment was followed by a one or two days interval. The period of the treatment lasted 30 days including the intervals. On the basis of the cytological examination 32 of the 304 (C_0) plants raised proved to be octoploid.

In the progeny of the octoploid plants the ploidy level was determined by various methods: the chromosomes were counted, pollen measurements taken and the number of chloroplasts in the guard cells of the stomatal pores determined. In the root tip and leaf primordium the chromosome number was determined by the well-known cold shock method. Chromosome examinations in the C_1 — C_5 generations were carried out, plant by plant, in the seed of mother plants varying in number from year to year. The number of chloroplasts in the stomata were determined by the method of BUTTERFASS (1960). As it is known there is a positive correlation between the ploidy level and the number of chloroplasts in the guard cells of the stomata, which can be successfully used to separate the tetraploid sainfoin plants from the octoploid ones. We studied, further, the number per mm^2 and size of stomata. The examinations were made on epidermis layers stained with a 1 percent silver nitrate solution. The pollen measurements were taken in preparations stained with carmine acetic acid. According to our previous investigations there is a considerable difference between the tetraploid and octoploid plants as to the meristemic tissue cells of the root tip, so the latter may provide a basis for the determination of the ploidy levels. The number per mm^2 and size of cells were therefore determined in this part of the growing tip. The examinations were performed in friction preparations made from the root tips of the germinated seeds and stained with carmine acetić acid. Microscopic investigations were made by means of a Nfpk2 research microscope equipped with a phase contrast fitting and phase objectives, with 400 × magnification.

In the tetraploid Mátra variety (control) and in the C_3 and C_5 generations 12 morphological characters were studied. Investigations were made partly in the field, partly with plants of the same age and development, raised in culture pots. The plants were examined for the following characteristics:

Leaf examinations

leaf weight
weight of a pair of leaflets
leaflet surface
length/width ratio of leaflets
member of leaflets per leaf

Examination of flower part

surface of standard
surface of wings
surface of keel
length of staminal column
length/width ratio of standard
length/width ratio of keel
length of floral axis

Results

Microscope studies. The major data of the two ploidy levels are presented in Table 1.

The somatic chromosome complement of the octoploid sainfoin is shown in Fig. 1. Besides the euploid forms numerous aneuploid (hypoploid) forms were found. Of them the form with 49 chromosomes was the most frequent, but plants

Table 1

Some data of tetraploid and octoploid sainfoin plants as determined by microscope

Characteristics examined	Ploidy level		Percentage proportion	
	4n	8n	4n = 100%	8n
Number of stomata per mm^2	108	58	100	53
Length/width ratio of stomata (micron)	22 : 17	30 : 20	100	136 : 117
Number of chloroplasts in the stomatal guard cells	14	27	100	192
Cell number per mm^2	1147	804	100	70
Length/width ratio of cells	25 : 17	37 : 22	100	148 : 129

Fig. 1. Somatic chromosome complement in octoploid sainfoin. Magnified: 90 × obj; 6,3 : 1 proj

with 52 and 54 chromosomes occurred as well. No hyperploid plants have been found so far; though hyperploid cells occurred in mixoploid plants. Plants containing cells with different numbers of chromosomes are summed up under the name: mixoploid.

The distribution of the ploidy levels in the C_1—C_5 generations is seen in Table 2. The table shows that the per centage of octoploid plants is relatively constant ranging between 86 and 95 per cent. Re-regulation to the tetraploid level practically does not occur, and even the percentage of sexaploid plants is negligible. The percentage of hypoploid plants, on the other hand, constantly increased from the C_1 generation onwards. Since their role — the consequence

Table 2

Distribution of C_1—C_5 generations according to ploidy level

Generation	8n %	6n %	4n %	Aneuploid %	Mixoploid %	n
C_1	90.28	3.95	0.09	5.42	0.26	2253
C_2	92.00	0.70	—	6.77	0.53	2836
C_3	88.37	0.57	—	9.58	1.48	2089
C_4	86.53	0.17	—	9.69	3.61	4573
C_5	95.00	—	—	4.65	0.35	3640

of their presence in the population and participation in the process of flowering — is unknown, all plants shown by the cytological examination to contain two or more aneuploid cells were discarded in the C_4 generation. It was supposedly the result of this that the proportion of aneuploid plants fell by some 50 per cent in the C_5 generation. A fluctuation in the frequency of aneuploids in the different young C-generations was also reported by AHLOOWALIA (1971) in rye-grass and by JARMOLYUK (1971) in sugar-beet. This is why the fertilization-biological and cytogenetical role played by the aneuploids in the population should be more thoroughly investigated in the future.

As to the other cytological properties, in the octoploid cockshead the cell measurements in the meristemic tissue of the root tip are larger and the number of cells per mm^2 lower. Its stomatal measurements are also larger, and the number of chloroplasts in the guard cells of the stoma is 90—100 per cent higher. Its pollen measurements exceed those of the tetraploid plants considerably, too, as in the latter the length of the pollen is 40 microns, its width 22.5 microns compared to the 55.3 micron long and 29.4 micron wide pollen of the octoploid. The pollen measurements found by us in tetraploid plants are perfectly identical with the results of GÖRGÉNYI (1955).

Morphological studies. The major morphological data of the two ploidy levels are shown in Table 3.

The octoploid sainfoin produced by the redoubling of the number of chromosomes has thicker and larger cotyledons. The first simple and the first compound foliar leaflets appearing almost at the same time in the two different ploidy level plants are also larger. There is, however, a shift in the phenophases; growth and development are slower in the octoploid form. Leaves of the fully developed octoploid sainfoin are of a darker green colour, and the leaf measurements exceed those of the tetraploid form. The weight of the leaf and leaflet as well as the surface of the leaflet are larger, the leaflet blade is thicker and the petiole stronger. The measurements of the flower parts are also larger in the octoploid form. The floral axis is longer, the standard, wings and the sur-

Table 3

Trends of some morphological properties in tetraploid and octoploid sainfoin plants

Properties studied	Ploidy level		Percentage proportions of 8n plants
	4n	8n	4n = 100
Leaf weight, g	1.0963	1.4068	128.32
Weight of a pair of leaflets, g	0.0883	0.1164	131.82
Leaflet surface, mm²	208.03	222.85	107.12
Length/width ratio of leaflets, mm	31.93 : 8,84	25.70 : 12,73	80.48 : 144,00
Number of leaflets per leaf	19.23	19.62	102.02
Standard surface, mm²	85.34	103.32	121.06
Length/width ratio of standard	11.54 : 8.17	11.78 : 10.32	102.08 : 126.31
Wing surface, mm²	5.36 : 5.36	6 : 6	111.94 : 111.94
Keel surface, mm²	79.46	92.66	116.61
Length/width of keel, mm	11.07 : 9.69	11.68 : 11.17	105.51 : 115.27
Length of staminal column, mm	10.52	11.50	109.31
Length of floral axis, mm	93.00	100.00	107.52

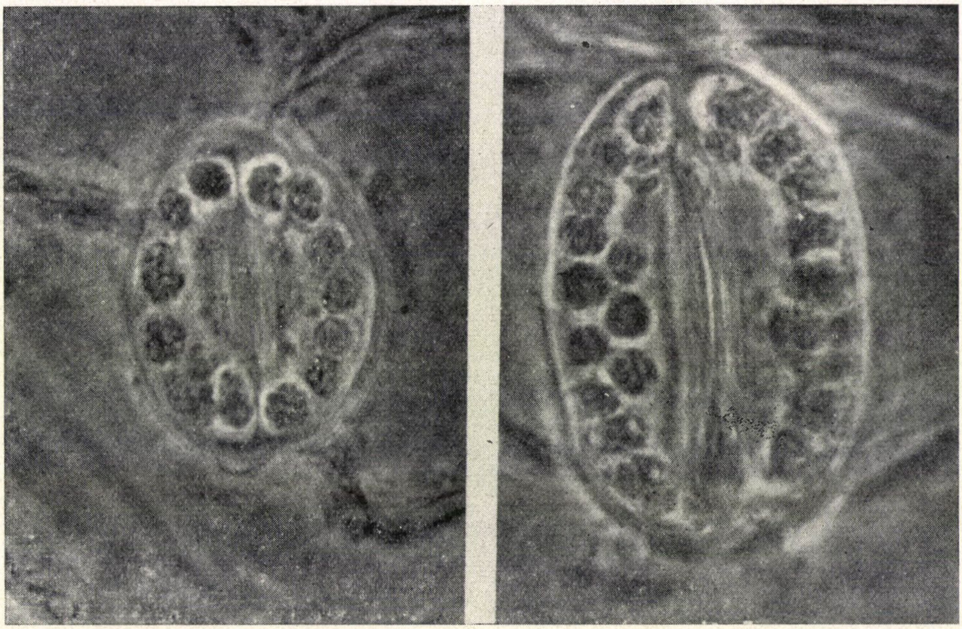

Fig. 2. On the left a tetraploid, on the right an octoploid sainfoin stomatal guard cell and the chloroplasts in them. Magnified: 90 × obj; 6,3 : 1 proj

face of the keel are larger, and the staminal column is longer too. The difference in the time of flowering is about six or seven days, that is, the octoploid plants begin flowering six or seven days later than the tetraploids.

Fertilization-biological and fertility conditions of octoploid plants as well as their fodder production data will be presented in a separate paper.

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PHYTOMASS STUDIES OF SALT PASTURES (ACHILLEO-FESTUCETUM PSEUDOVINAE) II

By

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Quantitative phytomass studies were carried out between 1969 and 1971 on a salt pasture (*Achilleo-Festucetum pseudovinae;* Ujszentmargita, IBP experimental area) and on an area excluded from grazing. The weight of aboveground parts was determined from cut samples, while that of underground parts from monolith samples after drying at 105 °C. The authors deal with the relationships between weather conditions and production, phenophases and productivity; also the quantity of production and the number of seeds counted in the soil. The productivity, turnover time and rate of production of aboveground and underground parts as well as of Festuca pseudovina are discussed.

Introduction

Information about the phytomass production study of a pasture situated next to the experimental area of our primary production studies carried out in the framework of the IBP has already been given in two publications (MÁTHÉ *et al.* 1967, MÁTHÉ—PRÉCSÉNYI 1970). The present paper contains the results of further three years (1969—71) of our continuous investigations.

Material and Method

To assess the weight of the aboveground phytomass, samples were cut from 20 × 10 cm² areas, from the pasture in eight, and from the area excluded from grazing in four replications (in 1968 a part of the pasture was fenced off as a meteorological station was being built there by the Meteorological Institute of the Kossuth Lajos University, Debrecen; on that area no grazing has taken place since then, and subsequently it will be referred to as "ungrazed area").

From the pasture, samples were taken only in spring, summer and autumn, while on the ungrazed area every month from April to October; the dates of sampling are presented in Tables 2 and 3.

The weights of the underground parts were determined after having been washed out of a monolith. The monoliths were only taken in the pasture, to a depth of 10 cm, in eight replications, from the same place and at the same time as the samples.

Weight data refer to materials dried out at 105 °C.

Results

Meteorological and phenological conditions. Weather was highly varied in the period examined. Table 1 shows some of the data obtained on the area for the growth seasons of the three experimental years, and placed at our dis-

Table 1

Meteorological data

Month	Mean temperature °C			Precipitation mm		
	1969	1970	1971	1969	1970	1971
April	10.0	10.7	11.6	37	70	16
May	18.6	14.3	18.6	4	92	68
June	18.7	19.1	18.8	38	99	77
July	21.3	20.4	21.0	42	93	33
August	19.7	19.8	22.0	58	161	42
September	16.1	(15,0)	14.1	7	(15)	45
October	10.5	8.7	8.5	12	5	19

posal by the Meteorological Institute of the Kossuth Lajos University which took part and co-operated in investigating the area under the leadership of the late Prof. D. Berényi.

In 1969 the low amount of precipitation throughout the whole vegetative period is especially conspicuous. Precipitation was also poor in comparison with the many years average; at Újszentmargita from April to October it was only 198 mm. May was warmer and sunnier, June cooler and cloudier than the average. The amount of radiation and the number of sunshine hours were high.

In 1970 the amount of precipitation was remarkably high compared to the previous year: from April to October 533 mm. Due to the cloudy weather the number of sunshine hours was low, there was a general lack of sunshine and radiation during the growing season.

In 1971 the amount of precipitation during the vegetative period — 300 mm — was considered favourable (unlike the national average); and so were the number of sunshine hours and radiation.

According to phenological observations made on 20—25 species, up till autumn, the individual phenophases in 1969 set in 1—2 weeks earlier than in 1970.

Quantity of production. Table 2 presents the dry weight data of samples taken from the *Achilleo-Festucetum pseudovinae* association of the ungrazed area. Of the plant species included only *Festuca pseudovina* — the main plant — and *Achillea collina* were weighed. The cryptogamous plants were also handled together under the name "moss"; this phytomass was mostly composed of a few moss species as e.g. *Ceratodon purpureus*, *Polytrichum piliferum*, *Camptothecium lutescens* etc., though some lichens (*Cladonia* sp.) also occurred in it, and sometimes *Psalliota campestris* too was found in the samples.

The data of Table 3 originate from the same association, but from the *at Újszentmargita*

Radiation gcal/cm^2			Sunshine hours			Relative humidity %		
1969	1970	1971	1969	1970	1971	1969	1970	1971
10 199	9 824	9.939	219	203	214	73	73	69
14 365	12 133	13 622	296	175	283	63	73	69
11 357	12 922	13 170	191	238	238	74	75	69
14 620	12 998	14 389	284	284	277	63	75	68
10 828	11 695	12 454	712	255	292	70	76	67
9 397	(9 000)	7 797	238	(185)	163	74		81
6 443	5 206	7 812	199	128	155	77	77	78

pasture. Species other than *Festuca pseudovina* were summed up as "other plants". As a consequence of grazing and trampling *Achillea collina* was of minimum weight; it was thus reasonable to include it in the other species. While in the spring the participation of mosses is considerable, later their weight is insignificant.

It is remarkable that in the pasture there are very high proportions of mosses in the spring, in certain samples they exceed the dry weight of *Festuca pseudovina*. On the ungrazed area the weight of mosses is much smaller.

Although 1970 was a rainy year, the aboveground spring phytomass of the pasture was less than in the previous year (Table 3). The phenomenon was not only connected with the intensity of grazing as the spring yield was considerably lower than in the previous year on the ungrazed area too (Table 2). It was probably an after-effect of the extreme lack of precipitation in 1969 that was still felt in the first half of the vegetative period. On the other hand, by summer and autumn the aboveground phytomass production on the ungrazed area far exceeded that of the previous year.

The significantly lower weight of phytomass on both areas compared to the previous year reflects the summer drought in 1971 (Tables 2 and 3).

It is worth mentioning as a striking phenomenon that on the ungrazed area the effect of discontinued grazing and disturbance caused by man is felt in the third and fourth year in that dicotyledons of higher growth habit (*Centaurea pannonica*, *Achillea collina*, *Plantago lanceolata*, *Sonchus asper* etc.) become more and more dominant and of the grasses *Agropyron repens* and *Lolium perenne* begin to gain ground.

As to changes in the underground phytomass, data are only available on the top 10 cm soil layer (Table 3). The phytomass was not sorted at all, the weight contains both living and dead underground plant parts. In the successive years nearly identical weights were obtained.

Table 2

Weight of living aboveground parts on the ungrazed area
(Mean values of 4 replications; g/2 dm^2)

Time of sample taking	*Festuca pseudovina*	*Achillea collina*	Moss	Total
1969				
19 April	5.7	0.3	0.9	6.9
20 May	5.7	2.0	0.1	7.8
20 June	3.1	2.5	0.05	5.65
16 July	4.0	2.5	0.1	6.6
21 August	5.7	2.3	0.02	8.02
25 September	5.0	1.7	0.0	6.7
22 October	3.9	3.1	0.1	7.1
1970				
16 April	3.0	1.2	0.2	4.4
16 May	3.8	2.9	0.1	6.8
11 June	13.1	1.8	0.1	15.0
16 July	9.3	2.0	0.1	11.4
21 August	7.0	2.8	2.1	11.9
17 September	10.0	4.8	1.5	16.3
23 October	11.1	4.0	0.4	15.5
1971				
15 April	6.9	2.4	0.9	10.2
14 May	8.7	1.0	0.4	10.2
15 June	6.9	0.0	0.4	7.3
15 July	6.2	0.6	0.2	7.0
17 August	4.1	0.05	1.0	5.25
16 September	7.6	0.5	1.3	9.4
10 October	8.6	0.0	0.7	9.3

Seed contained in the soil. In 1970 seeds were counted in some soil monoliths, a comparison of which is given in Table 4.

A comparison between the number of seeds per 0.1 m^3 (10 cm ×1 m ×1 m) and in a monolith taken from a nearby arable land shows that the number of seeds is considerably higher in the soil of the arable.

Seed counting data of a monolith taken from a closed *Artemisio-Festucetum* association are available for 1970. The lowest number of seeds were counted in this soil.

Productivity. Living aboveground parts. The productivity of the living aboveground parts on the ungrazed area is shown in Table 5. Overall conclu-

Table 3

Weights of living aboveground parts and of underground parts on the grazing area (mean values of 8 replications; g/2 dm² and g/dm³)

Time of sample taking	Living aboveground parts			Underground parts
	Festuca pseudovina	Other plants	Total	
1969				
19 April	3.2	4.0	7.2	10.5
20 June	1.8	0.0	1.8	11.5
25 September	1.4	0.1	1.5	10.7
1970				
16 April	2.1	0.8	2.9	10.6
11 June	2.5	1.0	3.5	10.9
17 September	2.3	0.3	2.6	10.0
1971				
15 April	2.8	0.2	3.0	14.7
15 June	1.3	1.5	2.8	12.1
16 September	1.6	0.1	1.7	6.2

Table 4

Number of seeds counted in a soil monolith
(number/0.1 m³; average of 4 samples)

Time of sample taking	*Achilleo-Festucetum*		Arable	*Artemisio-Festucetum*
	not grazed	grazed		
1970				
16 April	9.300	=	42.000	=
17 September	=	12.500	23.000	4.200

Table 5

Productivity of living aboveground parts on the ungrazed area
(g/m²/day)

Months	1969	1970	1971
April—May	1.5000	4.0000	0.0000
May—June	—3.3675	15.1850	—4.5310
June—July	1.8265	—5.1425	—0.5000
July—August	1.9720	0.5555	—2.6515
August—September	—1.8855	8.2035	6.9165
September—October	0.7405	—1.1110	—0.1785

sions can hardly be drawn from the Table, since, except the increase or stagnation in April—May, in the different years positive and negative values occurred in high variation at the various dates of sample taking. If in Table 5 only the April, June and September values are taken in consideration, then, as seen in Table 6, between June and September productivity can be said to be positive every year. For the whole period of investigation (April—September) the following results were obtained:

1969	1970	1971
—0.0630	+3.8385	—0.2595 g/m^2/day

Productivity on the pasture was studied on the basis of samples taken three times a year. In one case a positive, while in the other cases as well as on a yearly average a negative productivity was found (Table 6). This was due to grazing.

Table 6

Productivity of living aboveground parts and of underground parts

Aboveground parts
non-grazed area
(g/m^2/day)

Months	1969	1970	1971
April—June	—1.0650	9.2950	—2.3570
June—September	0.5650	0.6630	1.1250
grazed area (g/m^2/day)			
April—June	—4.2250	0.5250	—0.1600
June—September	—0.1500	—0.4550	—0.5900
Underground parts (g/dm^3/day)			
April—June	+0.0163	+0.0052	—0.0426
June—September	—0.0082	—0.0091	—0.0634

Productivity of Festuca pseudovina. The productivity of *Festuca pseudovina* can be seen in Table 7. In 1970—71 the pattern of productivity on the ungrazed area was very similar to that of *Festuca* studied in the *Artemisio-Festucetum pseudovinae* association of the nature conservation area (Table 7). Similarity is understood — of course — for the signs only, not for the extent.

Changes in the productivity of *Festuca* in the pasture only correspond to the productivity pattern of *Festuca* studied in the *Artemisietum* and on the

Table 7

Productivity of Festuca pseudovina
(g/m^2/day)
Non-grazed area

Months	1969	1970	1971
April—June	—2.0000	9.1800	0.0000
June—September	0.9650	—1.5800	0.3750
Grazed area			
April—June	—2.3333	0.7272	—2.5000
June—September	—0.4081	—0.3061	0.3225
Artemisio-Festucetum			
April—June	+2.6250	+2.4525	+0.5000
June—September	—0.6500	—0.5350	+0.6450

Table 8

Correlation coefficients between the productivity of non-grazed area (aboveground parts) and the changes of climatological elements
(1969—71)

	b	c	x
a	0.084	0.951	—0.029
b	=	0.018	—0.242
c		=	—0.043

a = changes in the monthly mean temperature
b = changes in the monthly amount of precipitation
c = changes in the monthly amount of global radiation
x = productivity
n = 18

ungrazed area in that from June to September productivity, here too, is negative, except the June—September period of 1971 when productivity was positive in all the three stands.

Relation of productivity to changes in the climatic elements. On the basis of the correlations found between the productivity of aboveground parts on the ungrazed area and changes in certain climatic elements (monthly mean temperature, monthly precipitation, monthly amount of global radiation, Table 8) a path analysis was made in order to determine the extent and direction of the influence the individual climatic elements exercised on the productivity in 1969—71 (Le Roy 1960, Précsényi 1971). The correlations were calculated on the basis of Tables 1 and 2. According to the results no change in the studied climatic factors had any decisive influence on the variability of productivity (Table 9). This is caused primarily by factors other than those included in the study. The result is, to some extent, easy to understand, as the stand is in the process of a recurrent succession, in a state of reorganization, and balance has not yet been re-established.

Underground parts. The changes in the weight of the underground parts are shown in Table 3 on the basis of the average of eight replications. This Table served as a basis for assessing the productivity (Table 6). In the first part of the period of investigation (April—June) a decreasing tendency was found from 1969 on. In 1969 productivity was still positive while in 1971 negative. In the second part of the period studied (June—September) a negative productivity appeared each year, with an increasing tendency from 1969 onward.

Table 9

Results of path analysis
1. path coefficients, 2. percentage share of the different factors

1.	P_a	P_b	P_c	P_e
Path coefficients	0.300	—0.261	—0.323	0.965

2. direct effects P_a	P_b	P_c	indirect	other P_e	total
9	7	10	—19	93	100%

P_a = path coefficient between changes in the monthly mean temperature and productivity,
P_b = path coefficient between changes in the monthly amount of precipitation and productivity,
P_c = path coefficient between changes in the monthly amount of global radiation and productivity,
P_e = path coefficient between other factors and productivity

Turnover time. When assessing the rate and time of material exchange in the living aboveground parts, samples taken three times a year were taken as a basis in both stands. On the ungrazed area the turnover time showed higher fluctuations (4.2 years) than on the pasture (2.7 years; Table 10). On the ungrazed area turnover is somewhat slower than on the pasture (2.5 and 2.1 years, respectively, on a 1969—71 average).

The turnover time in *Festuca* showed a high fluctuation on the ungrazed area (Table 10), while on the pasture its fluctuation was much lower (9.6 and 4.5 years, respectively). The turnover time of underground parts is very short and shows but a slight fluctuation (Table 10).

Rate of production. The samples taken in April showed in each case a lower percentage of the total annual production on the ungrazed area than in

Table 10

Turnover time of living aboveground parts, underground parts and Festuca pseudovina (year)

Year	Living aboveground parts		Underground parts grazed area	Festuca pseudovina	
	non-grazed	grazed		non-grazed	grazed
	area			area	
1969	5.5	1.3	1.1	2.2	1.7
1970	1.3	4.0	1.2	1.2	6.2
1971	3.5	2.3	1.7	10.8	1.8

Table 11

Production rate of living aboveground parts and of underground parts
(cumulative percentage values)

Year, month		Living aboveground parts		Underground parts grazed area
		non-grazed	grazed	
		area		
1969	April	35.9	68.5	32.1
	June	65.0	85.6	67.2
	September	99.9	99.9	99.9
1970	April	12.3	32.2	33.6
	June	54.3	71.1	68.2
	September	99.9	99.9	99.9
1971	April	37.9	40.0	44.5
	June	65.0	77.3	81.1
	September	99.9	99.9	99.8

Table 12

Production rate of Festuca pseudovina
(cumulative percentage values)

Year, month		Non-grazed area	Grazed area
1969	April	41.3	50.0
	June	63.7	78.1
	September	99.9	99.9
1970	April	11.5	30.4
	June	61.6	66.6
	September	99.9	99.9
1971	April	32.2	49.1
	June	64.4	71.9
	September	99.9	99.9

the pasture (Table 11). The situation was similar in June. The high percentage found on the pasture in spring can be explained with the fact that grazing hardly takes place — if at all — at that time of the year.

There is not much difference in the production rate of *Festuca* between the two areas (Table 12). In the pasture in spring somewhat higher percentages were found every year than on the ungrazed area. The situation was similar in June.

In the case of underground parts 30—45 percent of the annual production was found in spring. By summer the stand produced 65—80 percent of the total amount weighed till the end of September (Table 11).

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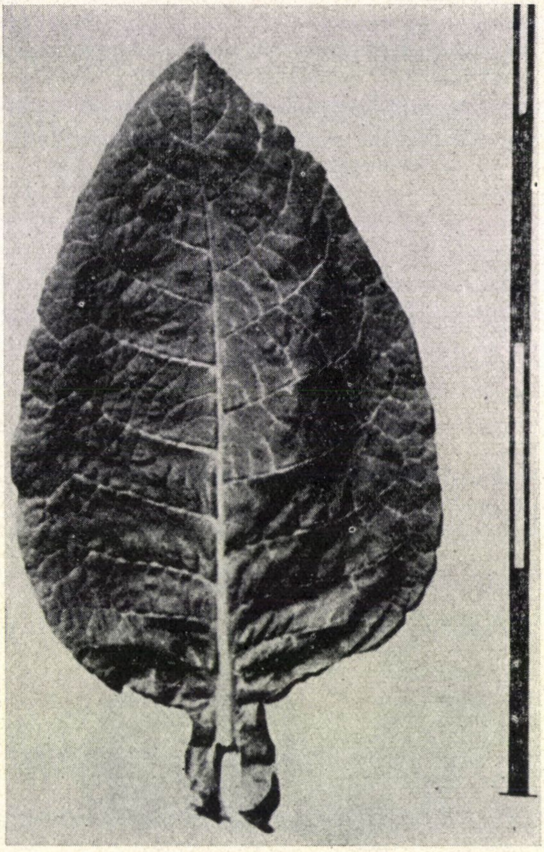
VARIA



TOBACCO VARIETY "KERTI"

Taxonomical place: *Nicotiana tabacum* L. var. *serotina* SCHRK.
Origin: an old local variety in Hungary
Beginning of breeding: not improved
State qualification: free variety licensed for sale
General characterization: rapid development, low nicotine content, productive, of light colour when dry, good cigarette material.
Morphological description:
- ***Root system:*** penetrating medium deep into the soil with plenty of adventitious roots.
- ***Shoot system:*** cylindrical shape
- ***Stem:*** 120—160 cm high, relatively thin, of 20—21 mm diameter at the base; an average of 28 nodes; light yellowish green colour, cylindrical shape; hairy.

Foliage: the 30—50 cm long and 22—27 cm wide leaves are arranged loosely on the stem, standing slanting upwards; the leaf blade is eggshaped, sessile, base slightly surrounding the stem, leaf apex bluntly acuminate. Leaves are of light yellowish green colour, leaf surface wavy, tissue fine, veins thin. The number of leaves may be as many as 26—28, though practically there are only 18—22 useful leaves. Leaf blade index: 1.38. Nicotine content: 0.6—1.2 per cent (MÁNDY 1952, MÓGER—SZÜCS 1966).

Inflorescence: loose, arched patulous polychasium of an average of 20 cm length, with 4—5 laterals and some 96 flowers.

Flowers: the funnel-like corolla tube emerges from a swollen calyx tube. The limbus of the corolla is almost pentagonal and slightly lobed. The colour of the corolla is pale pink with a star-shaped spot around the faux. The length of the flowers is 55—65 mm.

Fruit: 19—20 mm long capsule with a bluntly rounded tip, hardly opening when ripe. The number of ripe capsules may be as many as 90.

Seed: tiny, brown; thousand-grain-weight 7.2 centigram.

Biological characters:

Vegetation period: 80—120 days

Water requirement: among the Hungarian varieties it has the highest drought tolerance (KAPÁS *et al.* 1965).

Resistance to disease: satisfactory

Farm technology requirement:

Seeding: in the second half of March; planting in May.

Soil requirement: good condition sand, and sandy loam (MÓGER—SZÜCS 1966).

Productivity: high yielding; leaf yield: 9—12 q/cad. yoke (1 cad. yoke = 1.422 acres).

Region of cultivation: In Hungary Tolna and Nógrád counties.

Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen

GY. MÁNDY

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SOME QUESTIONS OF FLOWER ORGANIZATION IN SOUR CHERRY

The axial or foliar origin of the perigynous ovary and the surrounding hypanthium of sour cherry is a much discussed problem even today. We should like to clarify the organization of the simple reproductive growing tip of sour cherry thus providing data to these questions.

The flower organization of sour cherry and of the family *Prunoideae* was described by PAYER (1857) who performed morphological studies from the state of flower bud to the full

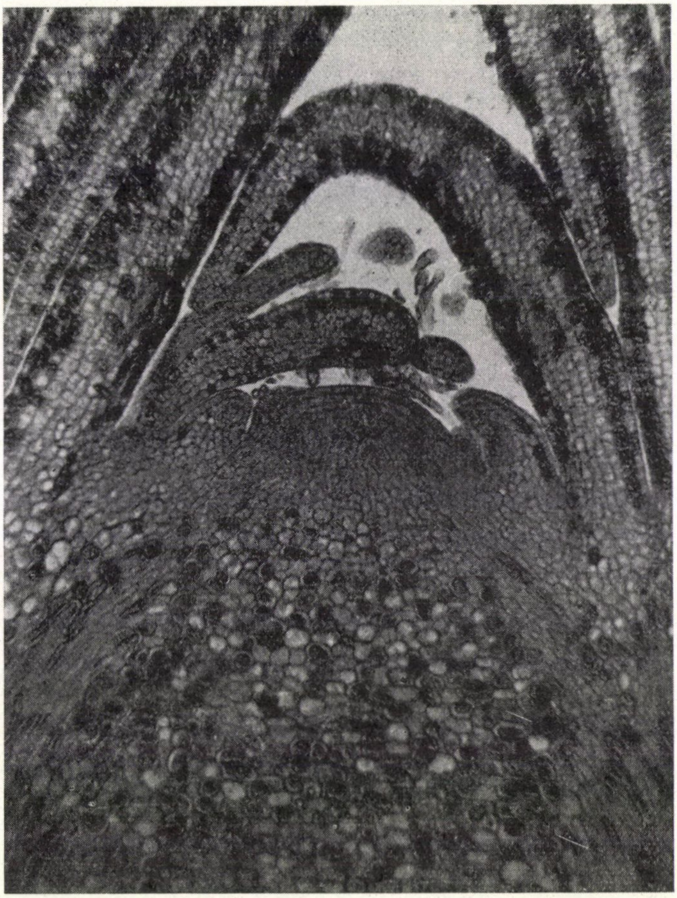

Fig. 1. Initial organization of the reproductive growing tip of sour cherry (obj. 10× oc. 8×)

development of the flower. He considered the hypanthium surrounding the perigynous pistil to be of toral origin. VAN TIEGHEM (1871), on the other hand, pointed out that the hypanthium developed by the growing together of the tissues of the calyx, petals and stamina. This opinion was confirmed by EAMES—MACDANIELS (1947), SCHÄPPI (1951), TAKHTAJAN (1959) and EAMES (1961). The development of the hypanthium was morphologically analysed by TROLL (1957) and TARNAVSCHI—MITROIU (1955) who — like Payer — suggested that it originated from the torus. STERLING (1964, 1966) studied a number of Prunus species but only dealt with the organization of the carpel in some detail. The abscission of the hypanthium and style in the cherry following flowering was described by LOTT—SIMON (1968), the structure of the fully developed flower and the course of fruit organization by RÁCZ—GRACZA (1972), the latter with histogenic investigations.

We began to collect the developing buds of sour cherry in the first days of June and repeated the collecting every two weeks until autumn. Next spring, from the beginning of bud bursting we continued collecting material, but at three days intervals as here development

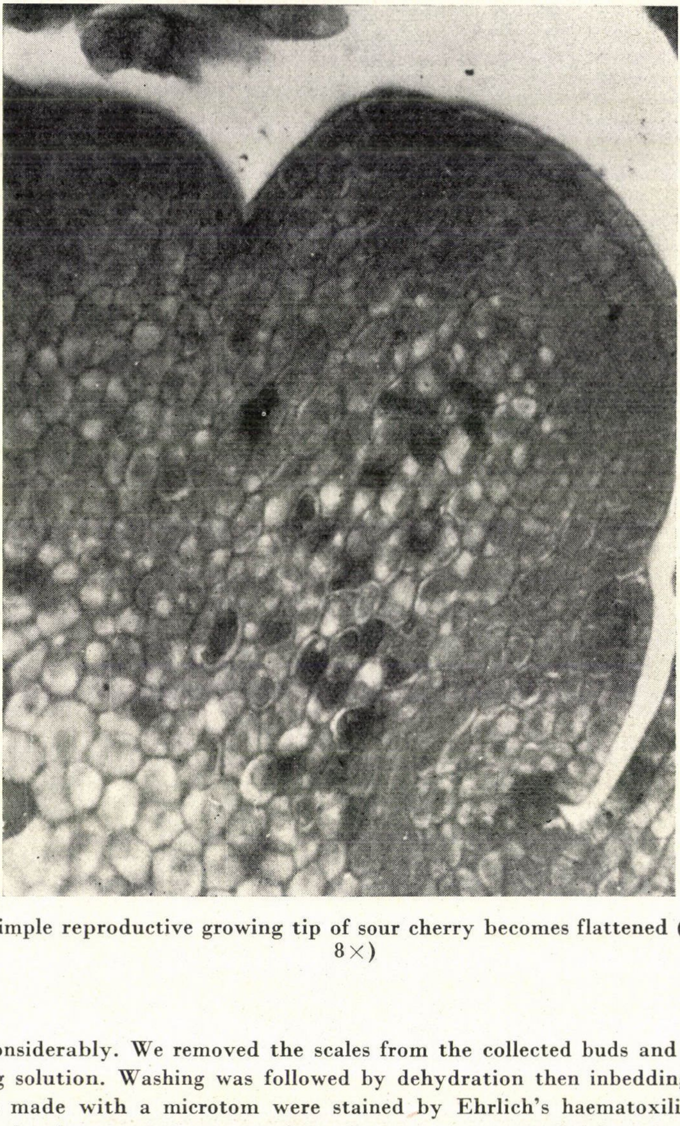

Fig. 2. The simple reproductive growing tip of sour cherry becomes flattened (obj. 40×, oc. 8×)

accelerated considerably. We removed the scales from the collected buds and fixed them in Bouin's fixing solution. Washing was followed by dehydration then inbedding in paraffine. Section series made with a microtom were stained by Ehrlich's haematoxilin. During the examinations the characteristic stages of development were recorded by microphotography.

On the spurs of the sour cherry a single leaf bud develops at the apex and several mixed buds below it. The small mixed buds differentiate first at a primordial stage with a vegetative character and so 3—5 supporting leaves appear above the scale primordia. In this vegetative phase of the growing tip the leaf primordia differentiate from the third tunic layer as a result of the cell division taking place by the insertion of periclinal then anticlinal walls. It is then that the bud primordium passes over to a reproductive phase. Above the developing small supporting leaf primordia the growing tip first broadens and somewhat flattens (Fig. 1). Further differentiation — unlike the earlier phases — takes place in the fourth tunic layer, superposed relative to the supporting leaf primordia. In this layer periclinal cell division begins in 4—5 cell diameters, the produced cells lift the superposed tunic layers and 3—4 small simple

Fig. 3. The concave reproductive growing tip of sour cherry with the sepal primordia at the edges (obj. 10×, oc. 2×)

reproductive growing tips appear. The initial organization of the reproductive growing tips begins in the middle of July.

The small reproductive growing tips at the tissue zone of the corpus considerably increase in number by periclinal and anticlinal cell division and develop in a rather short time into a 14—15 cell-row high and 22—23 cell-row wide hemisphere.

This occurs at the basal level of the developing reproductive growing tips, at the abaxial part. In the second tunic layer periclinal then anticlinal cell division begins which results in the development of α leaf primordia. Somewhat later, lower by a short internode, on the inner side that is in the adaxial part of the reproductive growing tip new cell divisions can be observed and the torosity of β bracteole appears. This suggests that with the appearence of the bracteoles the differentiation on the small developing reproductive growing tip at first can be considered vegetative.

After this the reproductive organization on the reproductive growing tip comes into, prominence. This can be observed in the vigorous cell division starting in the upper part of the

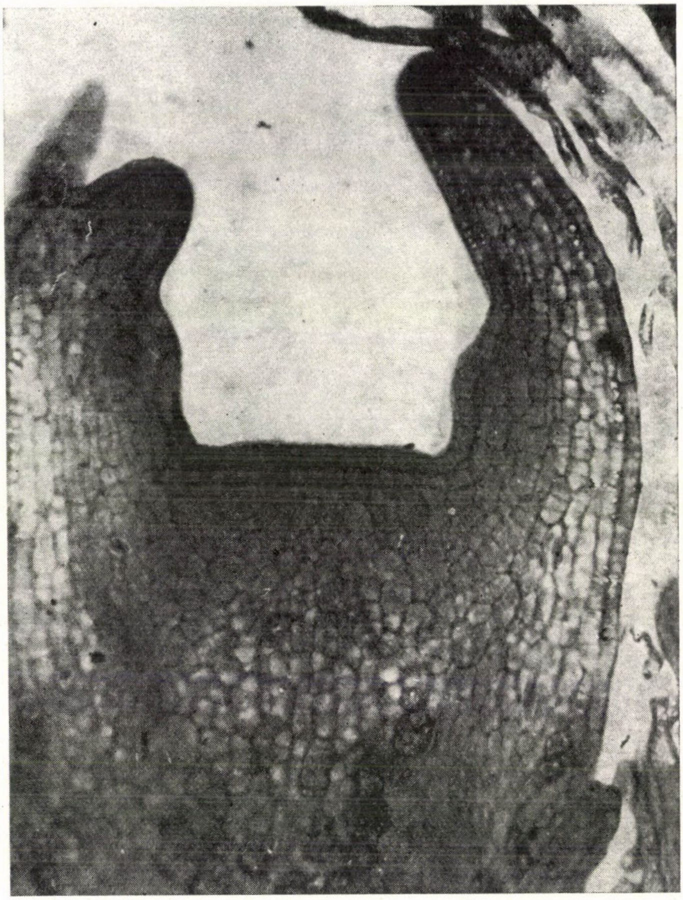

Fig. 4. On the inner side of the reproductive growing tip of sour cherry, below the sepal primordia the petal primordia appear (obj. 20×, oc. 8×)

convex reproductive growing tip, slightly periferially, as a result of which the surface of the reproductive growing tip rises at the edges and soon becomes level with the direct apical part. Thus in a second phase of development — so to say — the surface of the reproductive growing tip becomes flat and in a longitudinal section cup-like (Fig. 2). The new cell division activities also occur here, at the edge of the reproductive growing tip, in the second tunic layer, which results in the formation of five small protuberances: the primordia of the sepals (Fig. 3). The sepal primordia have hardly arisen when the tissue zone of the growing tip below them maintains its vigorous increase, namely with an intensive meristemic character toward the centre of the growing tip while in the outer part — at the border of the torus primordium and sepal primordia — with an intensive cell elongation. Thus the shape, and at the same time the surface of the reproductive growing tip changes. The flat growing tip becomes gradually concave. The further development of the sepal primordia only begins then; their edges join and they grow in length too.

Fig. 5. Two reproductive growing tips of sour cherry at different stages of development (obj. 10×, oc. 8×)

On the concave reproductive growing tip further differentiation occurs in this development phase of the sepal primordia, and is decidedly influenced by the shape of the concave growing tip. Namely, new cell division activities can be observed immediately below the sepal primordia, in the side-wall of the concave growing tip, again in the second tunic layer, and five small petal primordia develop arranged alternately to the sepal primordia (Fig. 4). For a while they grow inwards, then when their edges have joined and grown together begin to grow upwards. At an early stage the basal part of the petal primordia is in rather close contact with the sepal primordia, and in this initial phase petals and sepals grow together. At the same time the reproductive growing tip — while widening — becomes still higher at the edges. Its inner surface is no longer concave but square, with its vertical sides at right angles to the horizontal lower surface. Differentiation continues to take place in the side-wall part of the reproductive growing tip. Immediately below the petal primordia, in the second tunic layer, as a result of cell-division by periclinal then anticlinal walls protuberances of the upper stamina appear. Soon

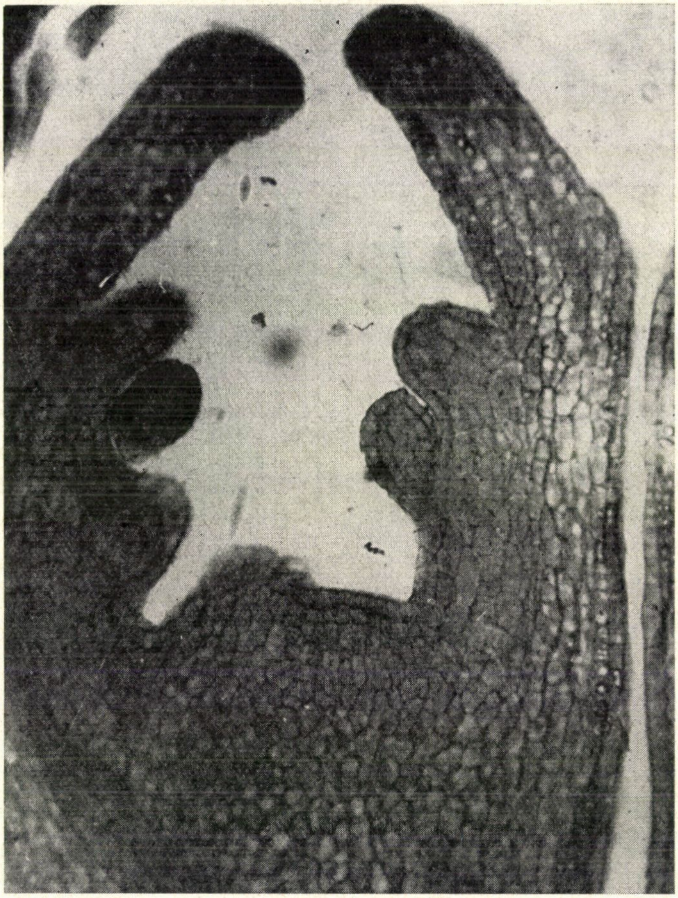

Fig. 6. On the reproductive growing tip of sour cherry the two staminal circles develop from above downwards (obj. 20×, oc. 8×)

after in the same tunic layer, in the side-wall part of the growing tip protuberances of the lower stamina appear as a result of a new activity of cell division (Figs. 5 and 6).

In the course of their development the stamen primordia first grow horizontally inwards, and only begin to curve upwards when the anther primordia have appeared and the filaments begun to develop. Filament primordia soon grow at their basal part to the joint tissue zone formed by the sepal- and petal primordia. In fact the tissue of the hypanthium develops by this, which is formed by the coalescent tissues of sepals, petals and filaments. The fact that the hypanthium is organized from the petals is confirmed later, at the fully developed stage of the flower, by studies on the vascular bundle. The 10 vascular bundles in the torus double when entering the hypanthium, and half of the 20 vascular bundles interweave the calyx and the outer stamina while the other 10 the petals and the inner stamina.

To return to the final stage of the differentiation of the reproductive growing tip we found that the primordium of the pistil initiated from the basal flat surface of the growing tip, the second tunic layer. The developing small carpel primordium shows a more intensive grow-

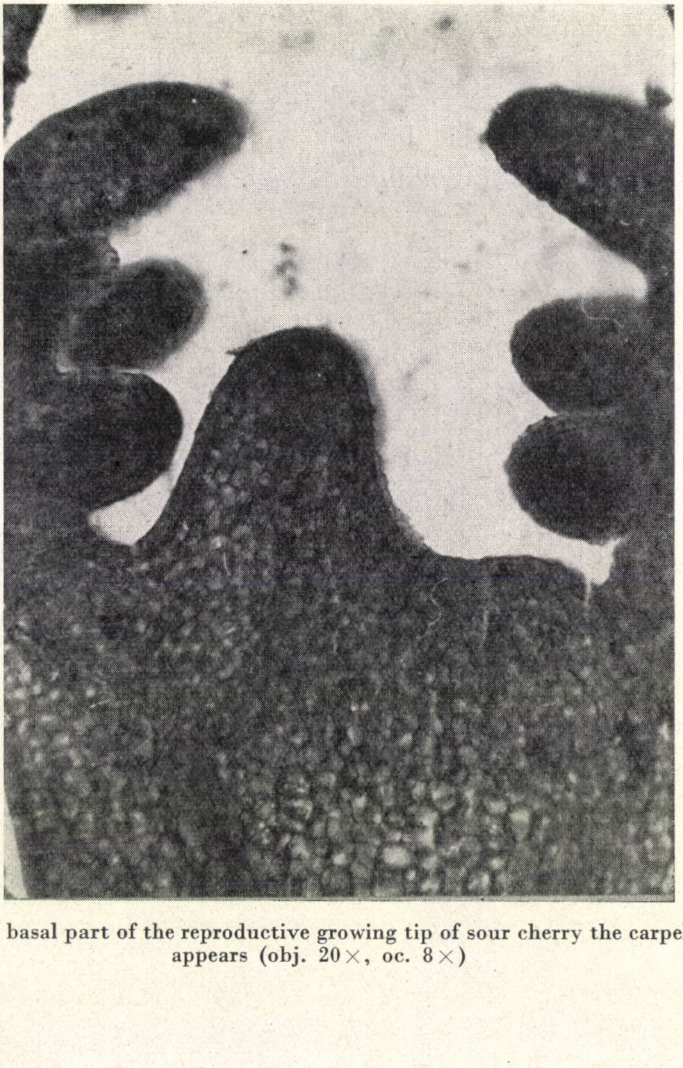

Fig. 7. At the basal part of the reproductive growing tip of sour cherry the carpel primordium appears (obj. 20×, oc. 8×)

ing along the main vein (Fig. 7), then it not only rises upwards at the edges but also widens in a semi-circle forward until the edges have joined and formed the cavity of the pistil (Fig. 8). The pistil developing from a single carpel is small in volume, so it develops freely in a so called perigynous position at one height with the hypanthium without touching its wall.

To sum up our investigations into the initial organization of sour cherry flower we may underline that as a consequence of the concave shape of the reproductive growing tip, petal primordia differentiate neither on the outer side (as e.g. on a convex growing tip), nor on the upper part, surface (as e.g. on a plate-like growing tip) of the growing tip, but on its inner side, and unlike the course of differentiation known so far (on a convex growing tip from below upwards, on a plate-like growing tip from outside inward) sepal-, petal- and stamen primordia develop from above downwards. Petal primordia arising in circles, nodes gradually becoming smaller and smaller result in the coalescence of these tissue zones and thereby the development of the hypanthium.

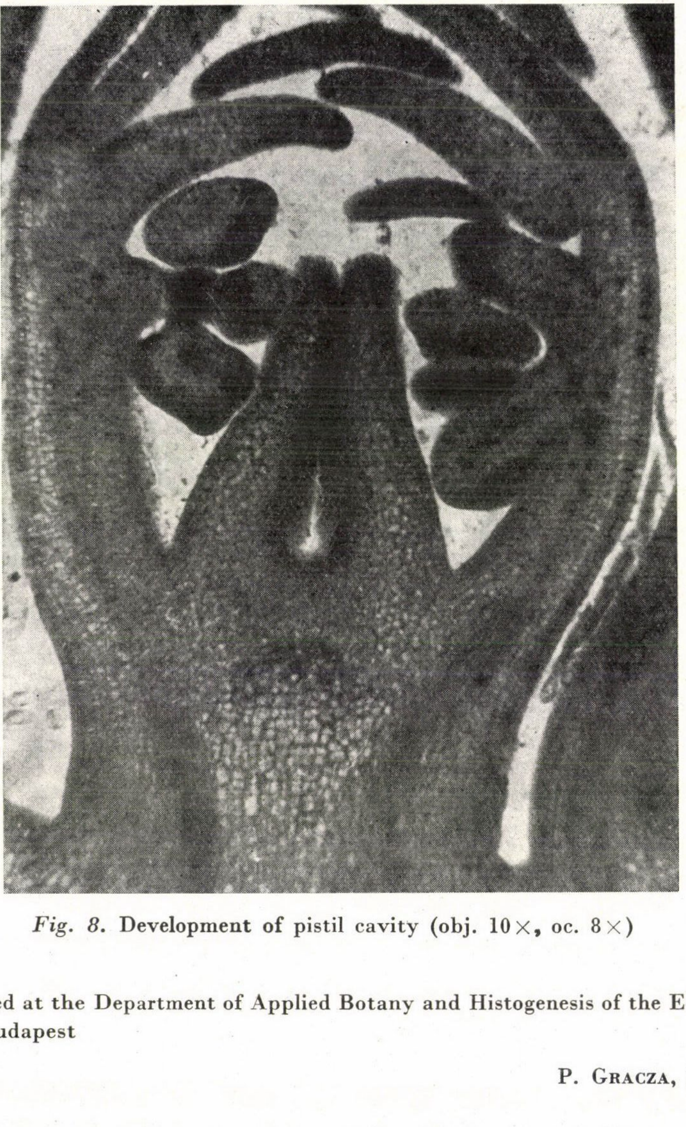

Fig. 8. Development of pistil cavity (obj. 10×, oc. 8×)

Prepared at the Department of Applied Botany and Histogenesis of the Eötvös Loránd University, Budapest

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NEW ASPECTS IN FRUIT-TREE PRUNING

("Sectorial fruit-tree pruning")

One of the methods of fruit-tree pruning: cut-back induces an increased regeneration wave in the cut back shoot formation at the expense of productivity. This can be avoided by omitting pruning, replacing it by bending the shoot, and by developing shoots from terminal buds (Fig. 1, III). Furthermore, the regeneration wave can be controlled and restricted by "sectorial fruit-tree pruning".

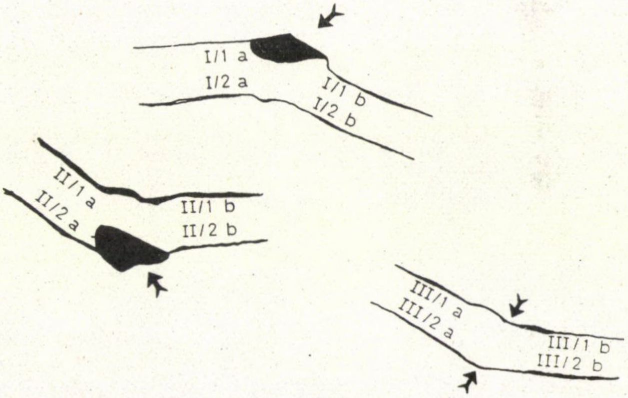

Fig. 1. Effect of cut-back on a slanting shoot formation. (Schematic radial section.). I. Drying up of the upper side as caused by cutting back to a lower bud (the dried up part hatched). II. Drying up of the lower side as caused by cutting back to an upper bud. III. Undisturbed material transport in a shoot developed from a terminal bud

The method is based on the utilization of sectorial disturbances caused in the material transport by pruning the fruit-tree (Brunner 1965, 1968, 1972). If, however, a slanting shoot is cut back to an upper bud, a one-sided drying up occurs on the low side (Fig. 1, II), and the apical dominance restored by the terminal shoot thus produced (Fig. 1, II/1 b + II/2 b) only restrains sprouting on the upper surface of the cut back shoot formation (Fig. 1, II/1 a), while on the lower surface (Fig. 1, II/2 a) this effect is not felt owing to the sectorial disturbance caused by the one-sided drying up in the sectorial material transport. Now, we must take into consideration that basipetal auxin transport, influenced by gravitation, produces an auxin concentration favourable for the development of buds on the upper surface of the slanting shoot, while on the lower surface concentration will be supraoptimal. In this way, on one hand, the restraining influence apical dominance exercises on the upper surface (Fig. 1, II/1 a) helps in preventing the development of water-sprouts, while on the other hand, the apical dominance shut out from the lower surface by the sectorial disturbance of material transport (Fig. 1, II/2 a) may promote the sprouting of buds on the lower surface, because in this way their hormone level, which owing to their position is higher anyway, will not increase further. Thus in the present case on the upper side the influence of apical dominance hinders the development of water-sprouts, while on the lower side this apical dominance shut out by the disturbance of sectorial material transport prevents the shoots from becoming bare. Pruning to an upper bud may, of course, result at the same time in the tzrminal shoot deviating from the direction of the slanting bough and approaching perpendicular. It is here that "sectorial pruning" has to be combined with bending, that is, tying the terminal shoot down in a slanting position.

In this context it must be noted that in the fruit-tree training carried out in the traditional way pruning is done just the other way round: shoots are cut back year after year to the lower buds in order to continuously ensure the slanting position of the bough. In this way, however, the restraining effect of apical dominance on sprouting is shut out by the one-sided drying up on the upper shoot surface (Fig. 1, I/1 a), where sprouting capacity is higher anyway, and an unfavourable water-sprout development is thus enhanced. The influence of apical dominance on the lower surface (Fig. 1, I/2 a) results in a further decrease in its sprouting capacity — which due to its position is reduced in any case — and the bough will eventually become bare.

In conclusion "sectorial fruit-tree pruning" when completed by bending is able to restrain water-sprout development on the upper surface of boughs of different inclination, and prevent the lower surface from becoming bare, thus rendering possible an increased productivity and larger producing surface nearer to the ground. "Sectorial pruning" can also be used during the crown rejuvenation and subsequent thinning of shoots.

Prepared at the Horticultural Research Institute, Budapest

T. Brunner

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HISTOLOGICAL CHARACTERISTICS OF LEAF- AND BARK DRUGS FROM NEOBRACEA VALENZUELANA (RICH) URBAN GROWN IN CUBA

Neobracea Valenzuelana is a wide-spread weed plant in Cuba. Attention has recently been called to its therapeutical value by Acuna Julian, Professor of Botany, who sent samples to Barcelona (Spain) for the purpose of plant chemical and pharmacological experiments. Investigations have revealed that this plant contains a substance of much stronger and more permanent hypotensive effect than Rauwolffia. These results make a pharmacognostic study of the plant necessary, primarily in respect of the leaf and bark which offer the drugs. The present paper, which forms the first part of our investigations planned with the plant, gives a description of the macromorphological and microscopic properties of the leaf- and bark drugs, which — in our opinion — can be used for identification.

The genus *Neobracea* was described in detail by Britton (Leon—Alain 1959). According to his description 5 species of the genus are endemic in the West Indies. Four of the five species live in Cuba, and one on the Bahama Islands. These species are xerophytes. They are at home in wind-blown places (zones exposed to the winds).

The species are: *N. angustifolia* Britt.: a native of the province Pinar del Rio, in soils of high Mg- and Ca content. (Cuba); *N. flowardii* Woods: grows in the mountains of las Villas; *N. Bahamensis* Britt.: is found on the coast of province Oriente; in the province Matanzas and on the Bahama Islands; *N. olxmanii* Urban: a native of the provinces Pinares and Pinar de Rio; *N. Valenzuelana* (A. Rich) Urban: can be collected in all provinces, but always in the vegetation zone called "Cuabales tejánsu". The latter species is a tree or bush which may reach a height of even 8 m, but is generally only 2 m high. It is a xerophyte of slow growth. It is a plant not cultivated, only growing wild. No data are available on its soil- and climatic requirements. Under tropical conditions it blossoms readily though not continually; it produces follicles.

In volume V. of Flora de Cuba compiled by Leon—Alain (1959) Rich and Urban describe the plant as follows: a tree or bush of 1—8 m height. Its leaves are oval, or oblong lanceolate, 2.5—6 cm long and 0.8—2 cm wide. There are elliptic leaves too, chipped off at the tip and pointed at the base. Its leaf surfaces are generally shiny. The flowers are pink. The receptacle is 2—8 mm, the pedicel 3—5 mm long; the corolla is 1.5—2 cm long. The follicles are thin and flexible, 9—15 cm long. It is an endemic plant growing everywhere in Cuba on a so called Kuabales soil.

For the present work the material was collected in the province Canossi from wild plants under the guidance of Prof. Luis Rojas Carballosa (Dept. de Farmakognosia y Fitoquimica, Universitas de la Habana). The test material arrived in a completely dry state and consequently the epidermis could not be removed from the leaves even after a lengthy soaking and special softening process (Sárkány—Szalai 1957). For this reason we made attempts with "Neatan" (Merck) a plastic film recommended by Lassányi (1971), which is generally used in thin layer chromatography to preserve and remove layers. In addition we tested, and ultimately applied successfully, the Hungarian make Plasztubol, a transparent plastic wound cover, which can be removed from the surface together with the epidermis.

These materials were used for the examination of both (upper and lower) leaf epidermes.

Superficial contaminations were removed with distilled water and the leaves dried on filter paper, then sprayed once or twice with Plasztubol or Neatan to obtain an appropriate thickness of layer. After drying the layer was removed and placed in a 1 : 1 ratio mixture of glycerine and water. To make the examinations easier we stained the layers with toluidine blue of various — though usually 2 per cent — concentrations.

Experience showed that the epidermis layers obtained with Neatan first had to be placed in water to become smooth before getting covered with water and glycerine.

Another histological testing method was: the application of a softening mixture: glycerine : alcohol : water for 24 hours. The material to be excised was then boiled in water for 1—2 hours. Having been prepared the sections were repeatedly boiled on a low fire with a 2 per cent KOH solution for the purpose of purification, then washed and covered by glycerine-gelatin (Sárkány—Szalai 1957).

Method of examining the pulverized leaf. Leaf powder previously ground and passed through a screen of 75 mm mesh was purified by boiling for 1—2 minutes in a 2 per cent KOH solution, washed thoroughly first with tap water, then with distilled water, and put in glycerine-gelatin for microscopic studies.

Method of examining bark sections. Sections excised from bark previously soaked in water for 24 hours were purified by boiling in a 2 per cent KOH solution, then washed and placed

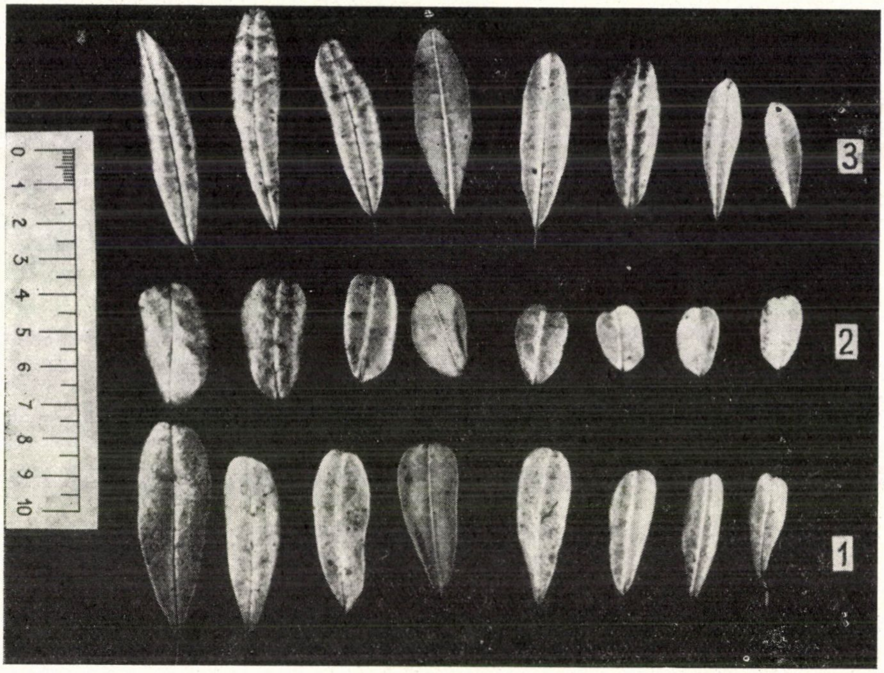

Fig. 1. Leaf types of *Neobracea Valenzuelana*

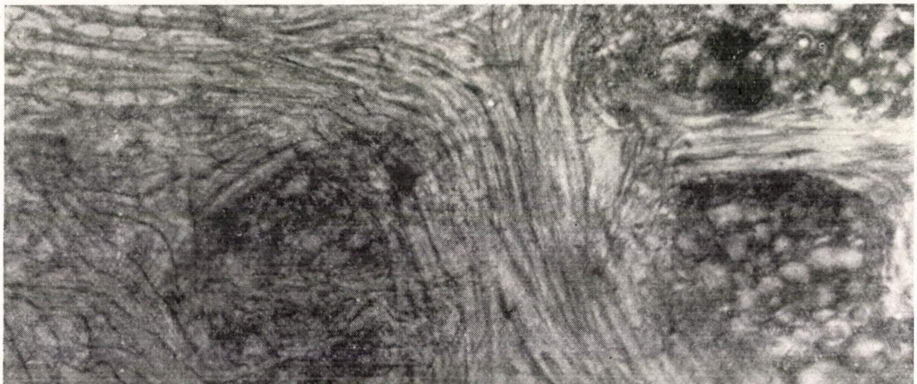

Fig. 2. **Preparation made with Neaten of the epidermis of the upper leaf surface (M = 8×4)**

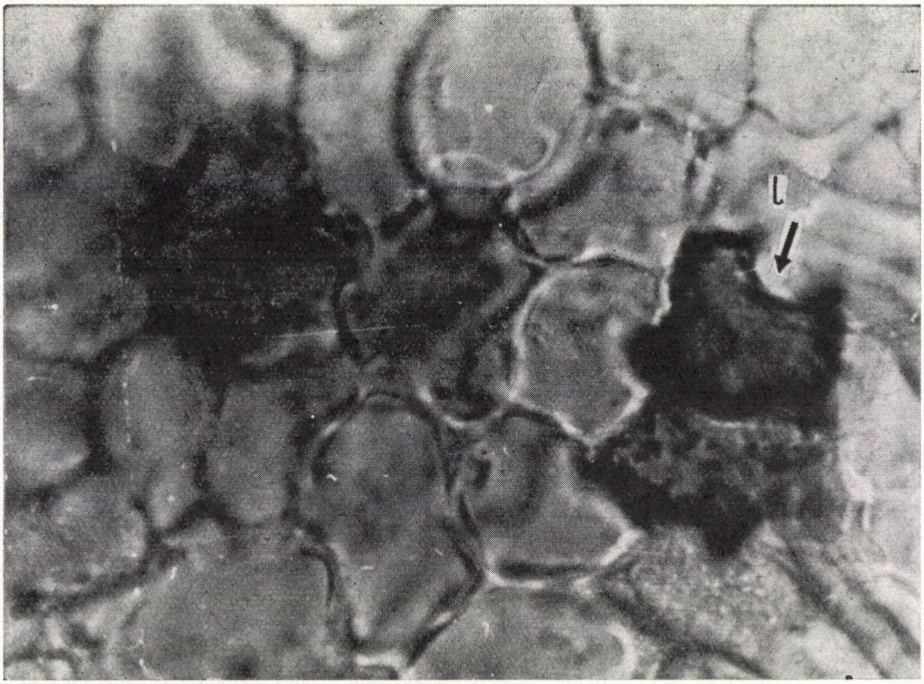

Fig. 3. Lactiferous vessels between the epidermis cells of the upper leaf surface (M = 42×4)

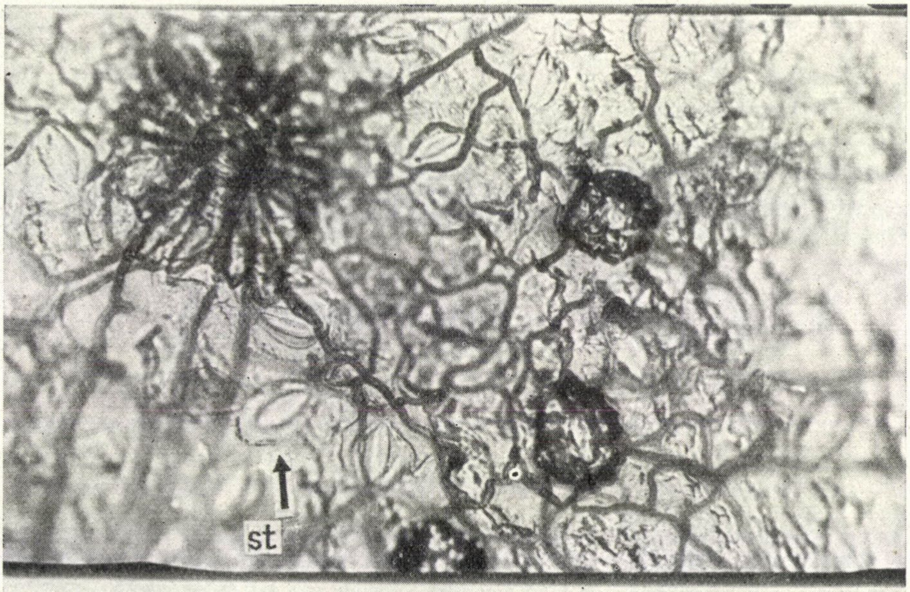

Fig. 4. Trichomes, crystals and stomata (st) in the epidermis of the lower leaf surface (M = 8×4)

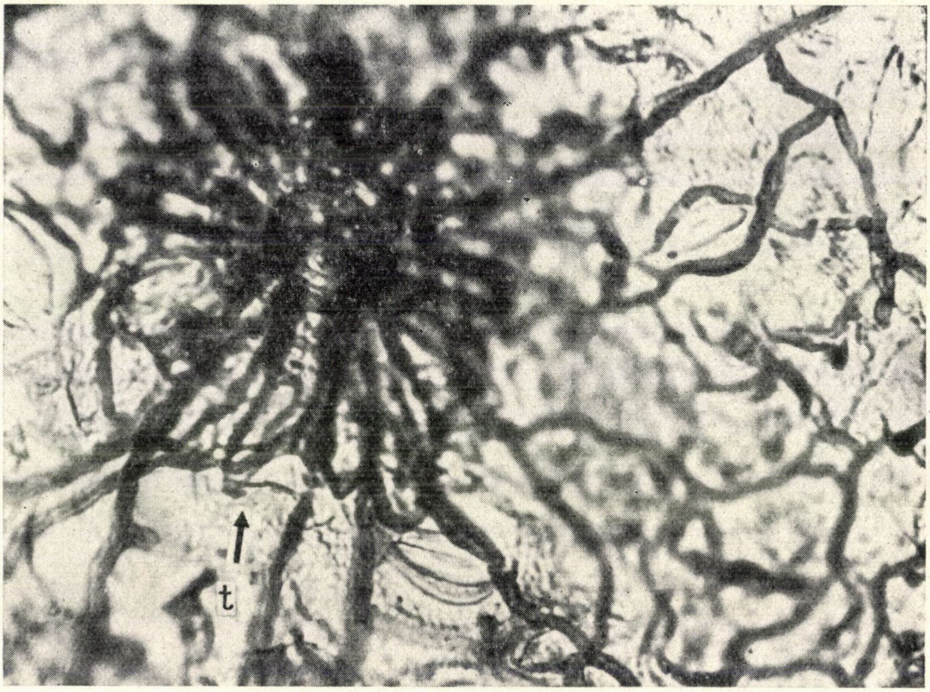

Fig. 5. Trichomes of characteristic structure, and lower surface epidermis cells with winding walls (t = trichome) (M = 42 × 4)

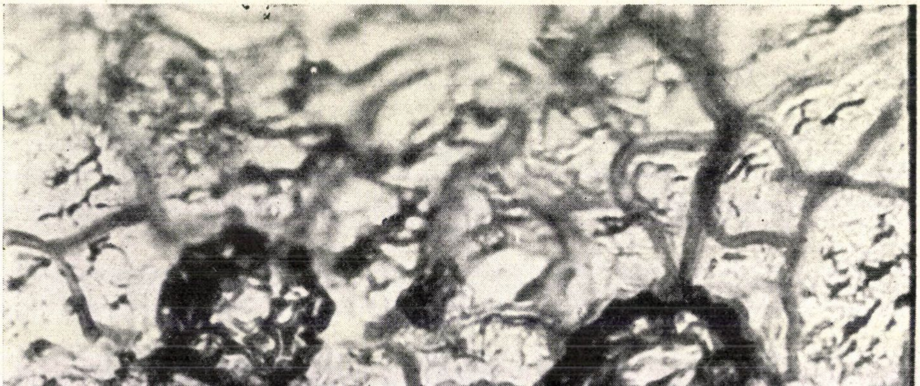

Fig. 6. Large rosettes in the lower surface epidermis (c = crystal) (M = 42 × 4)

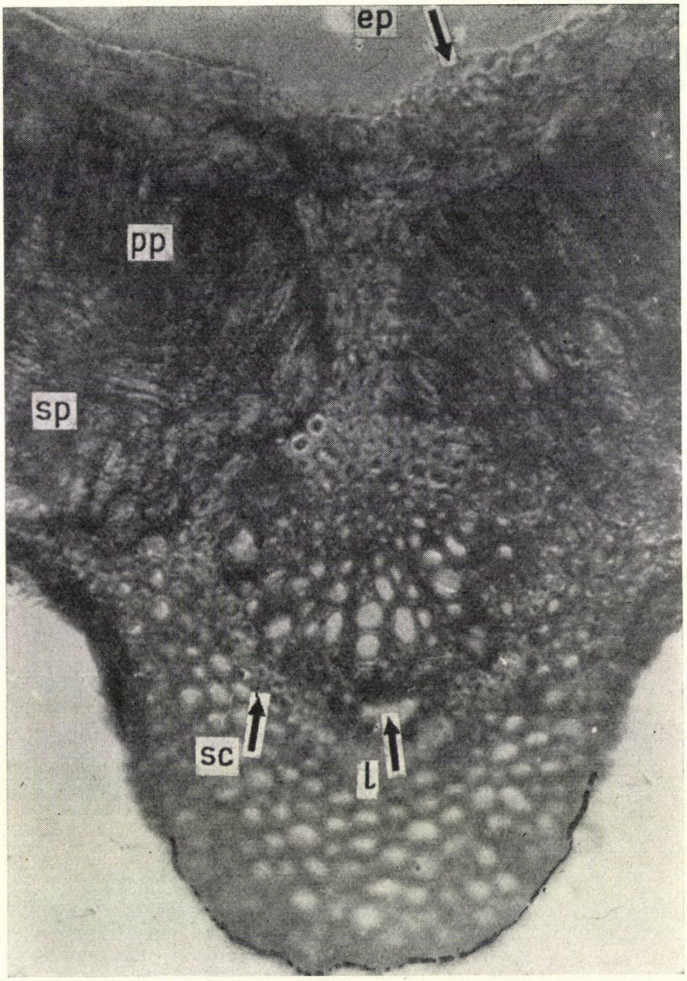

Fig. 7. Cross-section through the main leaf vein (ep = epidermis; sp = spongy parenchyma; 1 = lactiferous vessel; pp = palisade parenchyma) (M = 20×4)

in glycerine-gelatin. Having been purified with KOH and washed, some of the sections were stained with a 2 per cent solution of aquibus toluidin blue, then differentiated in water and covered in glycerine-gelatin for the purpose of examination.

Production of bark macerate in order to study the individual elements. The bark was broken up into pieces of the thickness of a match, the pieces placed in concentrated nitric acid to which potassium chlorate was carefully added, and the whole mixture slowly warmed up then boiled for 1—2 minutes in a gas-chamber. The material was washed several times, stained with a 2 per cent solution of toluidin blue, then washed again and placed in glycerine-gelatin.

Pulverous bark preparation. Produced in the same way as the leaf powder (see above).

Morphology of leaves. Leaves detached from the stalk and dried have 3 different forms: Some leaves have petioles while others are sessile leaves. The colour of the leaves is light green, sometimes with a red shade on the upper surface. The lower leaf surface varies from light green

Fig. 8. Sclerenchyma bundle surrounding the palisade parenchyma (M = 42×4)

to brown. The edges are rolled in, are stiff and fragile when dry. Leaf veins are pinnated and protruding on the lower surface (Fig. 1).

Shape of the leaf blade: Form 1.: asymmetrical, reversed egg-shape. Tip chipped off in a heart shape. Length 5.5 cm, width (at the widest point) 1.8 cm on an average; — smaller leaves are 1.4 cm long and 0.8 cm wide.—Form 2.: oval, with an asymmetrical base. Tip chipped off. The longest leaf is 3.5 cm, the smallest one 2 cm long. Width ranges from 2.2 to 1.2 cm. Form 3.: lanceolate, with asymmetrical base. Tip generally wedge-shaped. Length 6.4—2.6 cm; width 1.5—0.8 cm.

Histology of leaves. Epidermis on the upper leaf surface: In preparations made with Plasztubol the shape of the epidermis cells is not clearly seen, while in those prepared with Neatan they are readily distinguished (Fig. 2). Over the veins the epidermis is formed by long brick-shaped cells. The epidermis cells of the intervenia are mostly bluntly octahedral with slightly undulate walls (Fig. 3); between them are the guard-cells of the long laticiferous vessels reaching the surface. The trichomes are also characteristic appendages of the upper surface

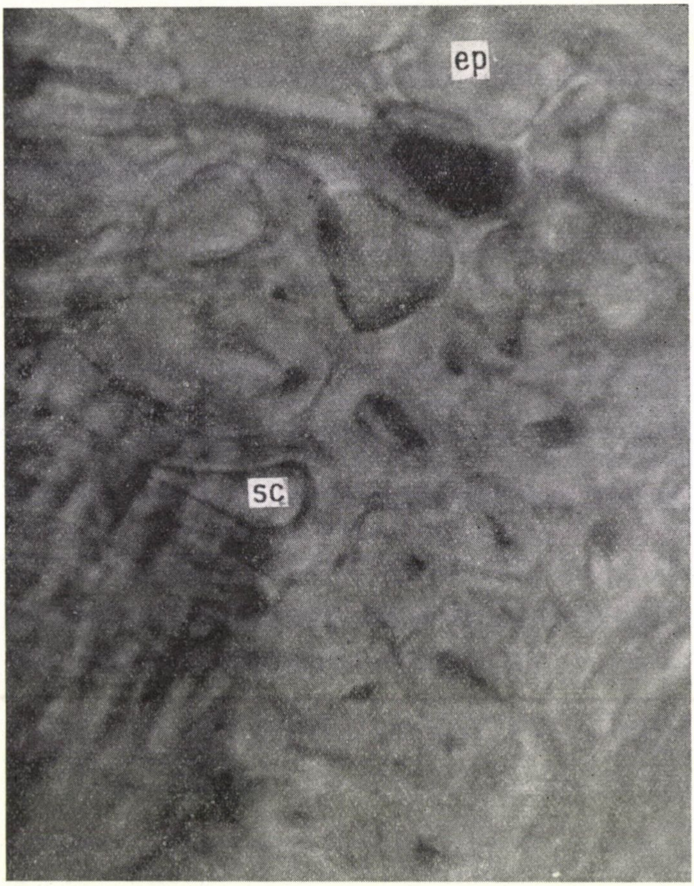

Fig. 9. Sclerenchyma bundle surrounding the palisade parenchyma one layer deeper than in Fig. 8. It can clearly be seen to be at right angles to the former one (sc) (M = 42 × 4)

leaf epidermis in *Neobracea Valenzuelana.* They are identical with those developing on the lower leaf surface. On a petiole composed of a number of cells, an average of 18—20 radially distributed spindle-shaped ray cells are found at right angles. Their surface is covered by a knitted cuticle. The trichomes are 78—84 microns in diameter. Enormous rosettes are found (Ca/COO_2) in certain cells of the epidermis, which with their rounded edges are often spherical. Their size is varied, the two diameters range between 28 and 71.4 and 34 and 57.8 microns respectively, on the basis of 20 measurements. On the upper leaf surface the epidermis has no stomata.

Epidermis on the lower leaf surface: can be easily studied with the Plasztubol technique too. The walls of the epidermis cells are winding, with simple pits. When measured along the longitudinal axis they are 74.8—34 microns long. Between the epidermis cells of the lower surface there are always stomata of around 24.9 per 1 mm^2 in number. They are surrounded by accompanied cells (two each) which form a type of stoma characteristic of the Rubiaceae. This in general is also characteristic of the family *Apocynaceae* (VERZÁR—PETRI 1971). The dimensions of the stomata — with the two guard-cells included — are: length = 30.6—35 microns, width = 27.2—34 microns. Both this and the illustrations show that the guard-cells surround

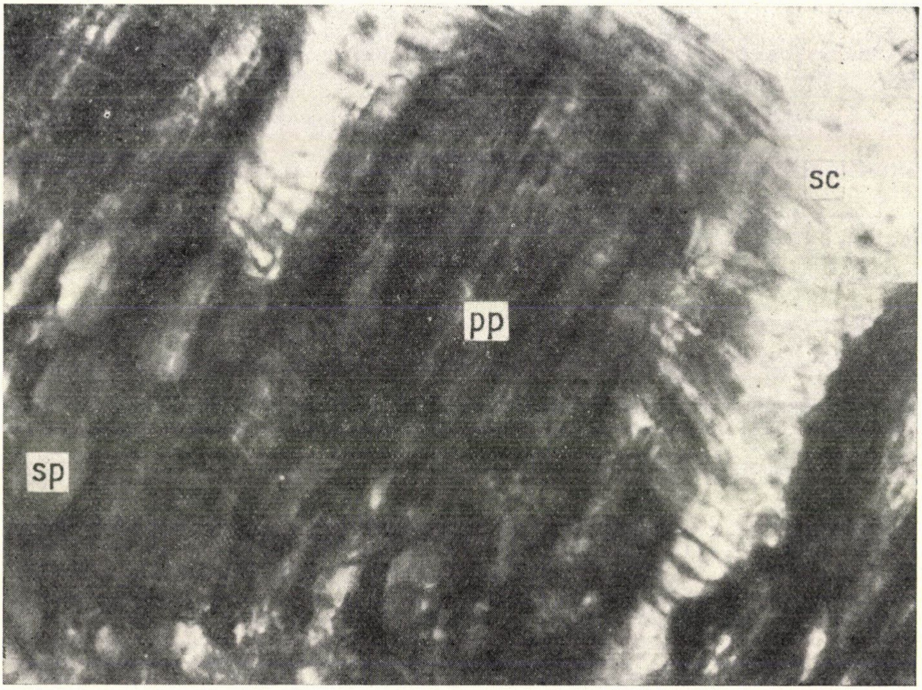

Fig. 10. Sclerenchyma columns in the cross-section of the leaf (M = 42×4)

a large oval stoma. The cells are usually narrow, just like the accessory cells. The trichomes look like multipetal open flowers, their description corresponds to that of trichomes found on the epidermis of the upper leaf surface. Their stalks consist of 2 or 3 cells. (Figs 4, 5, 6.)

Cross section of the leaf: dorsiventral leaf structure. The epidermis on the upper leaf surface consists of 3—4 rows of cells over the main leaf vein, and 1—2 rows away from the main leaf vein and on the ower leaf surface (Fig. 7). An elongated, closely set palisade parenchyma layer is found under the upper epidermis in 2—3 cell rows. Often the innermost layer of the upper leaf surface epidermis is, in fact, a thick-walled sclerenchyma layer of hypodermis character (Fig. 8), which is connected with the fibres above the vascular bundles (Figs 9, 10). These fibre bundles take up two directions, at right angles to one another, like parquet strips (Figs 9, 10), which gives the leaf extraordinary strength. They have a dome-like structure, and between them is found the assimilating mesophyllum which under the palisade parenchyma is completed by a relatively dense spongy parenchyma producing small intercellular spaces. The tissues of the leaf are closed by the epidermis of the lower surface.

In the cross sections of the leaf it can be clearly seen that the stomata and the trichomes are slightly sunken into the surface of the epidermis.

The main leaf veins sharply protrude on the upper leaf surface. They are surrounded by a sheath of sclerenchymatic fibre bundles. Their structure is bicollateral. The vascular tissue is crossed by a one cell-row of medullary rays at intervals of 8—10 cells. The phloem consists of sieve-tubes, accessory cells and bark parenchyma. The xylem consists of the following elements: tracheae arranged in rows, tracheids, a little metatracheal parenchyma and wood-fibres. Lacticiferous vessels are found on the outer borders of both the fibrillating parts of the phloem (Figs 7—11). Under the main bundles towards the epidermis of the lower leaf surface there are

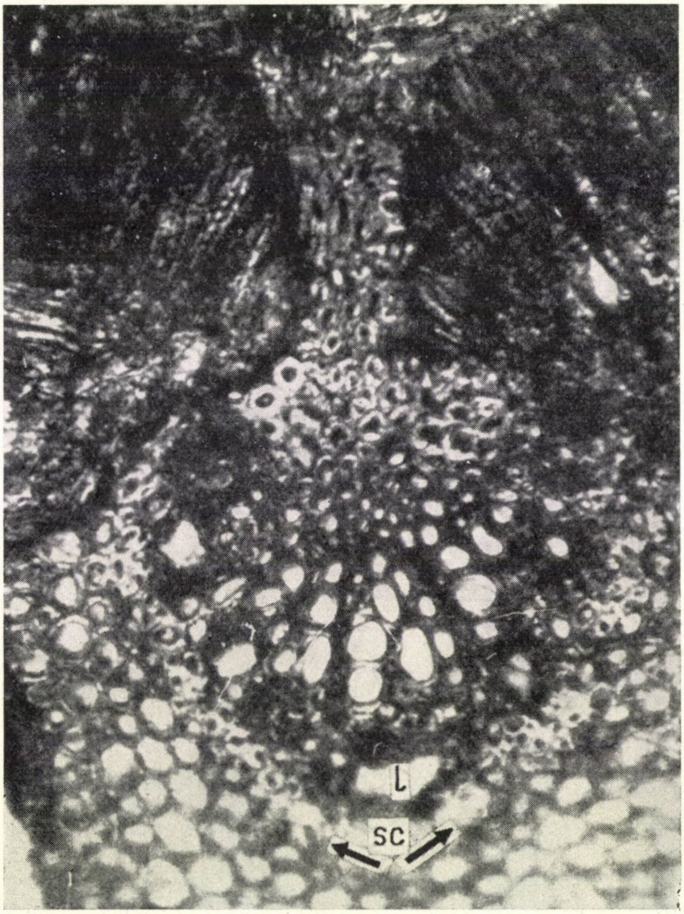

Fig. 11. Structure of the main leaf vein (sc = sclerenchyma; l = lactiferous vessel) (M = 42×4)

7—8 cell-rows of further izodiametric parenchyma. The cells are 13.6—23.8 microns long and 6.8—24.4 microns wide.

Examination of the pulverized leaf. The powder passed through a screen of 75 mm mesh is of green colour, characteristic spicy odour and bitter taste.

Trichomes, crystals, stoma fragments, vein parts and even some octahedric intervenial parts with the ends of veins are readily identified in the powder (Fig. 12).

Examination of the cortex. Morphology. The drug consists of 5—15 cm large pieces of bark with tubular structure and uneven surface covered with rhitidome. Their colour is greyish brown, their surface uneven, wrinkled. The surface of the fracture is fibrous; the inner part is lighter and has a smoother surface (Fig. 13).

Cortex macerate. The preparation mostly consists of thick-walled, evenly thickening narrow fibres with pointed tips (f) Fig 14); here and there disintegrated sclereids (sc) are found.

Parenchymatic elements are found in the macerate either in large coherent groups, or only occasionally.

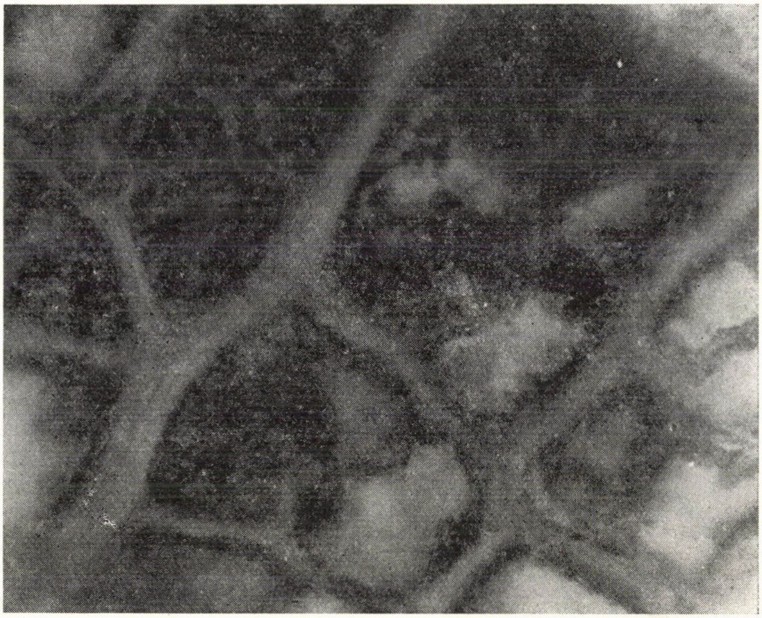

Fig. 12. Leaf pulver with vein-islets (M = 20 × 4)

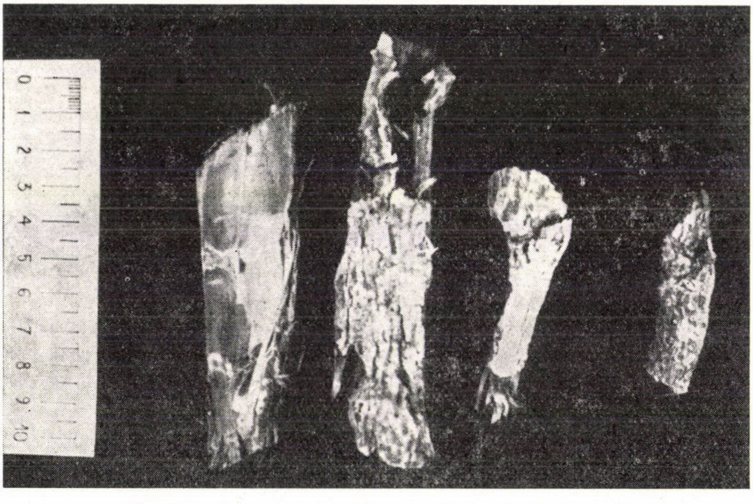

Fig. 13. Macroscopic picture of the bark drug

Fig. 14. Bark macerate (f = fibre; sc = sclereid) (M = 20×4)

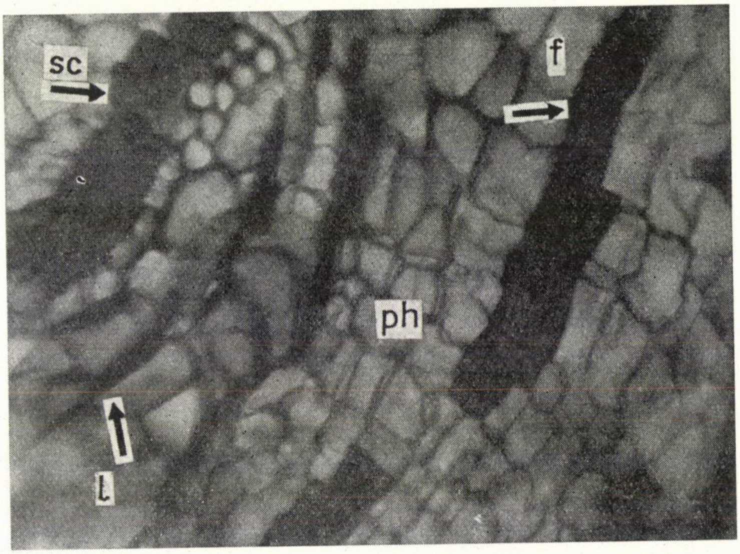

Fig. 15. Rhitidome. F = fibres; ph = phellogen; sc = sclereids; 1 = lactiferous vessel (M = 42×4)

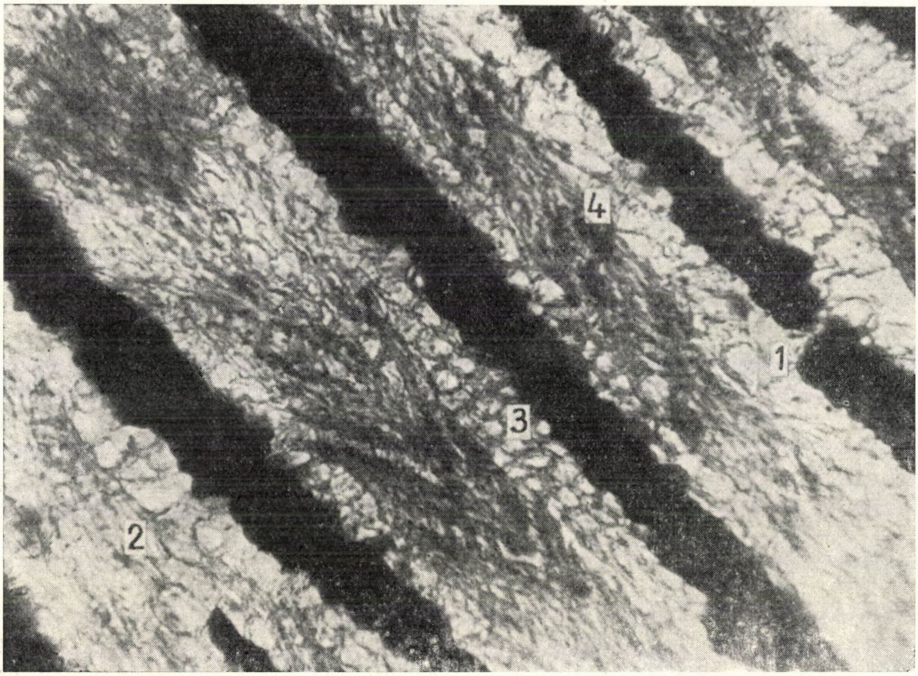

Fig. 16. Tissue structure of the secondary bark; the dark stripes consist of hard phloem (fibres); 1 = parenchyma break-through, one cell-row; 2 = parenchyma break-through, multi-cell-row; 3 = crystalliferous locular fibres; 4 = laticiferous vessel (M = 8 × 4)

Anatomy of the cortex. The rhitidome contains phellogen in several layers, which has a dipleuric action. The cells of the strip-like phellom are brick-shaped, loose in the centre of the surface. The cells of the phelloderma are radially arranged with undulate walls; between them sclereids can be observed grouped in rows; the latter are already characteristic elements of the phloem. The characteristic picture of the rhitidome is completed by phloemic fibre bundles and regularly alternating soft phloem layers (Fig. 15).

The bulk of the drug is composed of the secondary bark (Fig. 16). Hard and soft phloem layers alternate in it. The hard phloem consists of 4—5 rows of very thick-walled fibres, and — mainly on the borders — of sclereids, which are 34—61 microns thick, highly stainable with toluidin-blue, thus capable of lignification to a considerable degree.

They are interrupted sometimes by a 1—2, sometimes by a 10—20 cell-rowed parenchyma layer (1). The parenchyma groups are not always immediate continuations of the medullary rays.

The soft phloem is of heterogenous structure (2). On the border of the hard phloem, at a width of 2—3 cell-rows it consists of locular fibres containing crystals (3) (Fig. 17a, b). Lactiferous vessels alternating with readily collapsing sieve-tubes, accessory cells and a higher lumen phloem parenchyma arranged in a row can be observed in it (4). This often stripe-like pattern makes the drug easy to identify by microscope.

Pulvis. In the pulverized bark fractures of sclereids and groups of crystalliferous supplementary fibres are the most typical. Here and there parts of lactiferous vessels can also be observed (Fig. 18).

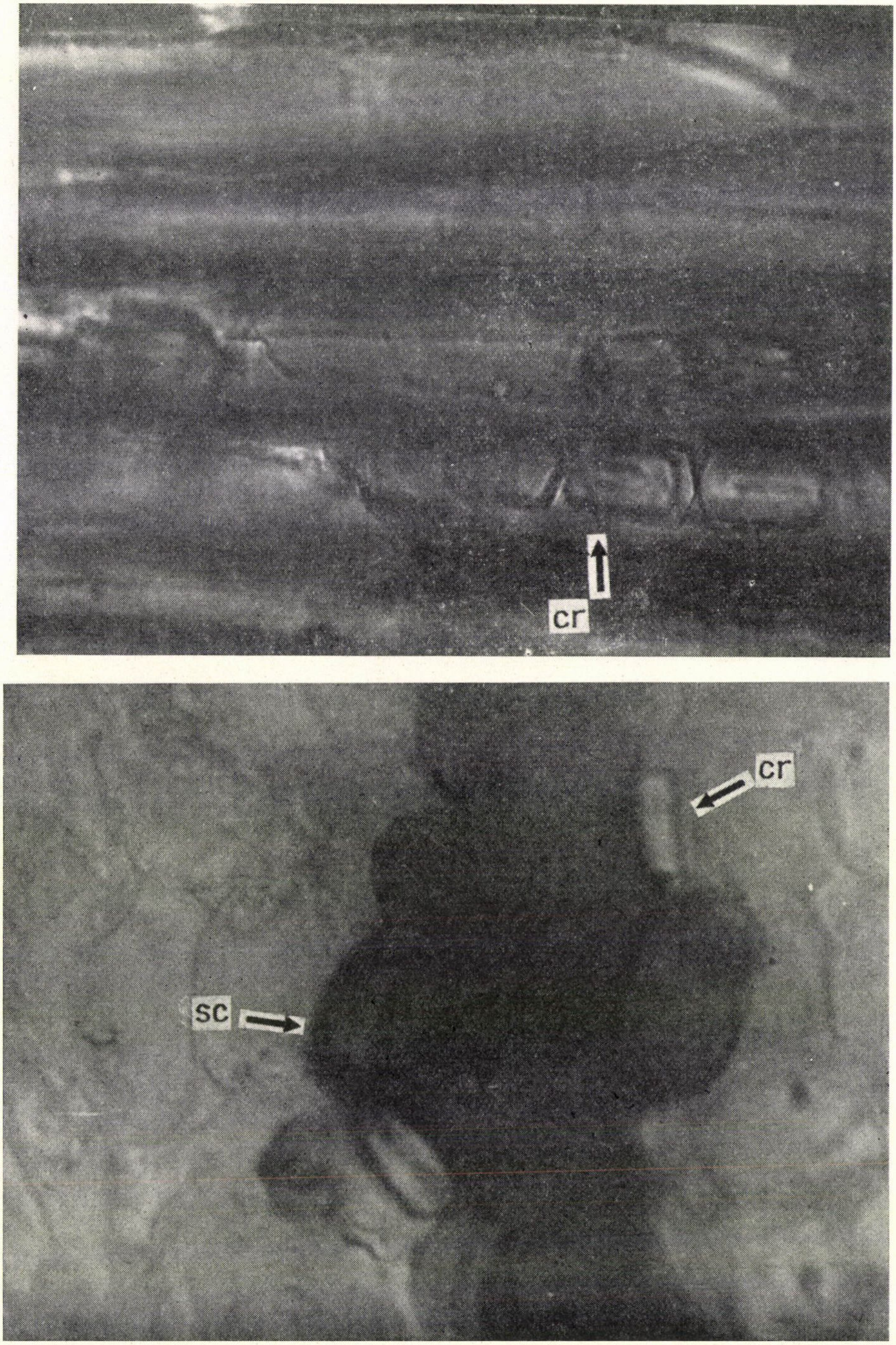

Fig. 17. Longitudinal section of crystalliferous locular fibres; 17/b = cross-section of the same (M = 42×8)

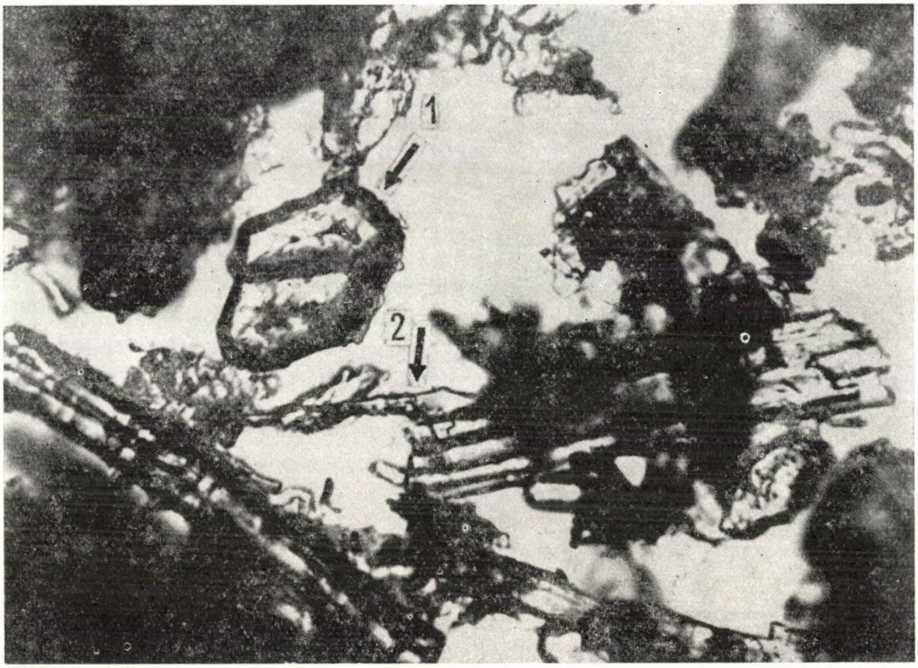

Fig. 18. Bark pulver. 1 = sclereids; 2 = crystalliferous locular fibres (M = 20×4)

Prepared at the Institute of Pharmacognosy, Semmelweis Medical University, Budapest.

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SEED TRANSMISSION EXPERIMENTS OF POTATO VIRUS M AND POTATO VIRUS S IN LYCOPERSICON SPECIES

Since the first successful virus transmission experiments carried out with seeds of plants (e.g. *Phaseolus vulgaris* L., *Echinocystis lobata* [Michx.] Torr. et Gray) infected by bean (common) mosaic virus ($^*/_*$: $^*/_*$: E/E : S/Ap) and cucumber mosaic virus (R/1 : 1/18 : S/S : S/Ap) (cf. Reddick—Stewart 1918, 1919; Doolittle—Gilbert 1919) numerous phytopathogenous viruses have been proved to be transmitted by seed. According to two recently published summarizing works (reviewed by Bennett 1969, Horváth 1972a) more than sixty plant viruses are transmissible by seed. In the seed transmission of viruses an important role is played by *Lycopersicon esculentum* Mill. As it is known the tobacco mosaic virus (R/1 : 2/5 : E/E : S/$_*$; Milinkó 1956, Taylor *et al.* 1961, Broadbent 1965), the potato spindle tuber virus (*/$_*$: */$_*$: */$_*$: S/Ap, Benson—Singh 1964, Singh 1970), and some NEPO-viruses (e.g. arabis mosaic virus, R/1 : (41 : S/S : S/Ne; the tomato black ring virus, */$_*$: */$_*$: S/S : S/Ne, Lister—Murant 1967, Murant—Lister 1967) are transmissible by tomato seed. In the course of our experiments performed in the last several years we found that in addition to *Lycopersicon esculentum* Mill. and *Lycopersicon chilense* Dun. other *Lycopersicon* species were also susceptible to infection by potato virus M and potato virus S (Horváth 1971, 1972b). Considering that the *Lycopersicon* species studied in most cases either responded to the infection latently or with hardly visible systemic symptoms it is especially important to find out whether potato virus M and potato virus S are transmissible by the seeds of infected but often seemingly perfectly healthy plants.

Our experiments were thus aimed at pointing out the possibility for the seed transmission of potato virus M and potato virus S with various *Lycopersicon* species (*L. esculentum* Mill. cv. *Red Cherry*, *L. glandulosum* Muller, *L. hirsutum* Hum. et Bonpl., *L. humboldtii* Dun., *L. peruvianum* [L.] Mill., *L. pimpinellifolium* [Jusl.] Mill., *L. pyriforme* Dun., *L. racemiflorum* Dun. and *L. racemigerum* Lange).

The methods used in the experiments were the following. The infection of plants inoculated with potato virus M and potato virus S was checked serologically and by test plants (cf. Horváth 1971). A proportion of tomato seeds collected from virus infected plants and dried was floated for 10 minutes in a 2 percent solution of NaOH, washed repeatedly in distilled water, then dried and planted in seed boxes. Another proportion of the seed was planted in seed boxes untreated. During the experiment fifty seeds per virus and tomato variety were planted. The seedlings were first pricked out, then transplanted into clay pots of 10 cm diameter, containing a soil mixture of peat, sand and compost. The virus infection of the plants was checked serologically and by test plants after the second and third week respectively.

We obtained negative results in both experiments. Thuson the basis of the results of our investigations it can be established that potato virus M and potato virus S transmissible both mechanically and by aphids could not in our case be transmitted with the seeds of various infected *Lycopersicon* species.

Acknowledgements

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SOME QUESTIONS OF FRUIT ORGANIZATION IN CHERRY

Some details of fruit organization in stone fruits belonging to the family *Rosaceae* have already been cleared up (ADDOMS *et al.* 1930, DORSEY—PORTER 1932, LILLELAND 1932, LILLELAND—NEWSAME 1934, RAGLAND 1934, TUKEY 1937, TUKEY—YOUNG 1939, LEE—TUKEY 1942, EAMES—MACDANIELS 1947, PÉNZES 1957, ESAU 1960, WILLING 1960, LOTT—SIMONS 1966, SÁRKÁNY—SZALAI 1966). According to some authors the kernel begins to develop soon after fertilization (RAGLAND 1934, ESAU 1960). Differences in the descriptions of the mesocarp and endocarp raised the idea of studying the organizational relations of these two tissue zones.

For the purposes of investigations fruits in five different stages of development were collected in the Botanic Garden of the Eötvös Loránd University. The individual stages of development were brought into connection with the size of the fruit. The test material collected was fixed in Bouin solution, embedded in paraffin, then section series were prepared from it with a Reichert-type microtom. Within the subject of fruit development only the organization of the fruit wall will be discussed here.

The pistil developing from a single carpel is joined with a broad base to the basal part of the hypanthium, the floral axis proper (Fig. 1). At the border of the pistil and floral axis a slight retraction of a width of 4—6 cell-rows can be observed. At the level of the retraction, as in the uppermost node of the flower, bundles running upward from the floral axis join and form a bundle sheath. It is from this that bundles interweaving the pistil start: a large dorsal bundle, two seed primordium bundles on the ventral side and next to them two wing-bundles as well as several vegetative bundles between the wing-bundles and the dorsal bundle.

At the height of the cavity of the ovary the pistil slightly widens then narrowing again continues in the elongated style. The stigma is of 50—55 cell-rows in diameter. Under the epidermis covering its surface the elongated cells running parallel with the longitudinal axis are vacuolized and contain green chloroplasts. Farther in more procambial bundles are found running up to the stigma. The wide tissue cylinder running upwards from the base in the centre of the style still has a pronounced meristemic character. This meristemic layer produces the several cells high papillation of the stigma.

Fig. 1. Longitudinal section of the cherry flower with pistil and hypanthium wall (obj. 2,6× oc. 3,2×

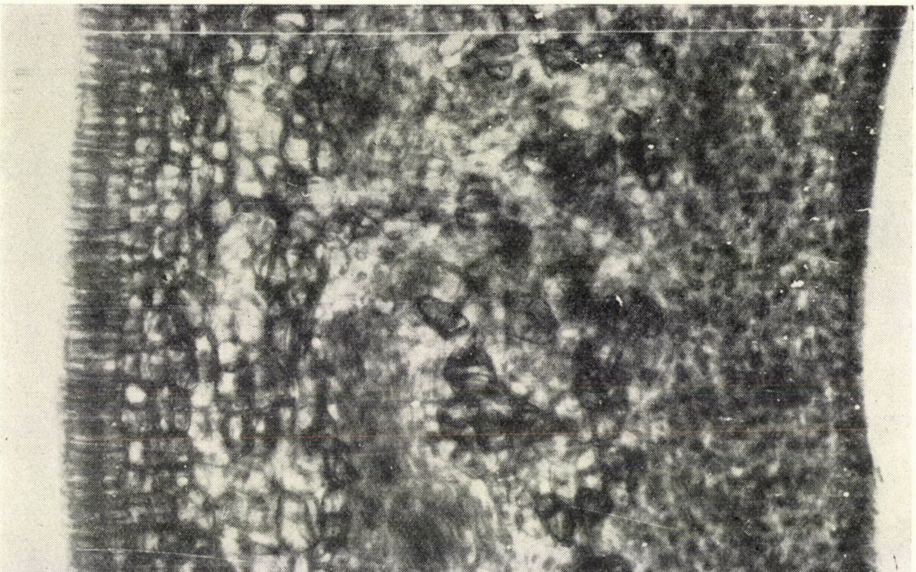

Fig. 2. Cross-section of the pistil wall; the inner tissue zone is still highly meristemic (obj. 10×, oc. 4×)

Cells of the epidermis covering the ovary show an intensive radial elongation and have large central nuclei. Certain cells have undergone a cell division by anticlinal walls. The mesophyllum of the pistil consists of 20—25 cell-rows on the dorsal side and of 30—35 cell layers on the ventral side. This high degree tissue growth is produced by the joining of the edges of the carpels, the development of the placenta. The two ovules lying one above the other rise from this area. Now the tissue characteristics seen in cross sections made from the mesophyllum of the pistil will be discussed in detail (Fig. 2). Immediately under the epidermis the cells are already in the process of stabilization, cells in the 2—3 subepidermal cell-rows are slightly elongated parallel to the surface. The cells contain chloroplasts. Farther in the cells become isodiametric. Between larger cells containing chloroplasts smaller readily stained meristemic cell groups are seen. This tissue zone of the mesophyllum is some 15—16 cell-rows wide and provides the bulk of the wall in the fully developed pistil. Inward from this, as far as the inner epidermis a meristemic tissue zone consisting of 8—10 cell-rows follows which is continued upward in the style. Its cells are small, in cross section generally triangular or square, easy to stain, with a large nucleus. This meristemic tissue zone forms one-third or a quarter of the fruit wall. The cells of the inner epidermis generally are square, sometimes elongated at right angles to the surface, or flattened parallel to it; in every case of highly meristemic character. At the basal part some stiff shiny hairs rise from the inner epidermis around the placenta too. The differentiation of the vascular tissue system has already started. In the external tissue zone, which is already becoming stabilized, the more intensely stained bundles of the procambial bundle system appear at a distance of 5—6 cell layers from the external epidermis.

In the next, second stage the young developing fruit is some 6 mm long and of 2—2,5 mm diameter. The hypanthium still surrounds the growing fruit, but at the basal part the abscission layer already begins developing. 3—4 days after flowering an important change has occurred in the tissue conditions. In the outer part of the fruit wall the ratio of stabilized and meristemic cells has changed compared to the former state: the bulk of this tissue part is formed by meristemic cell groups brought about by intensive division. In this part of the fruit wall the number of cell layers has become 19—23 (Fig. 3). From here inward the tissue zone showing a fully meristemic character from the beginning maintains this property and through a highly intensive cell division increases the number of its cell-rows to 25—30. In the immediate vicinity of the inner epidermis the cells elongate parallel to the surface (Fig. 4).

At the third stage of development (Fig. 5) the young fruit is 10 × 8 mm. Through the action of the abscission layer the hypanthium was separated at the border of the fruit and fruit stem. At this stage the processes of stabilization dominate, especially in the outer zone of the fruit wall. Cells of the single cell-layer epicarp developing from the epidermis begin to elongate parallel to the surface. At the same time in the underlying 2—3 cell-rows the outer and inner tangential walls of cells elongated parallel to the surface — i.e. tangentially — thicken and an intensive vacuolization starts. In the meantime there has been intensive growth in the outer tissue zone of the mesocarp through the meristemic activity already described and the number of its cell-rows has become 30—35. The cells have in a short time grown to a great extent and show intensive vacuolization. Cells stabilized at the pistil stage and those newly produced but already similarly vacuolized can be distinguished quite well, because in the earlier stabilized cells the chloroplasts can be seen even at this stage.

The separation of the mesocarp and endocarp occurs at this stage of development. The inner meristemic tissue zone of the mesophyllum of the pistil — as it has been mentioned — showed an intensive growth but no remarkable differentiation at stage II.

At this stage a further growth (to 50—52 cell-rows) in the inner tissue of the fruit wall occurs on one hand, and the size of cells increases on the other, while inside the cells other cytological changes can be seen. The cytoplasm occupies the space along the cell-wall round the large central vacuole. In cells adjacent to the inner epidermis a secondary thickening of the cell-walls

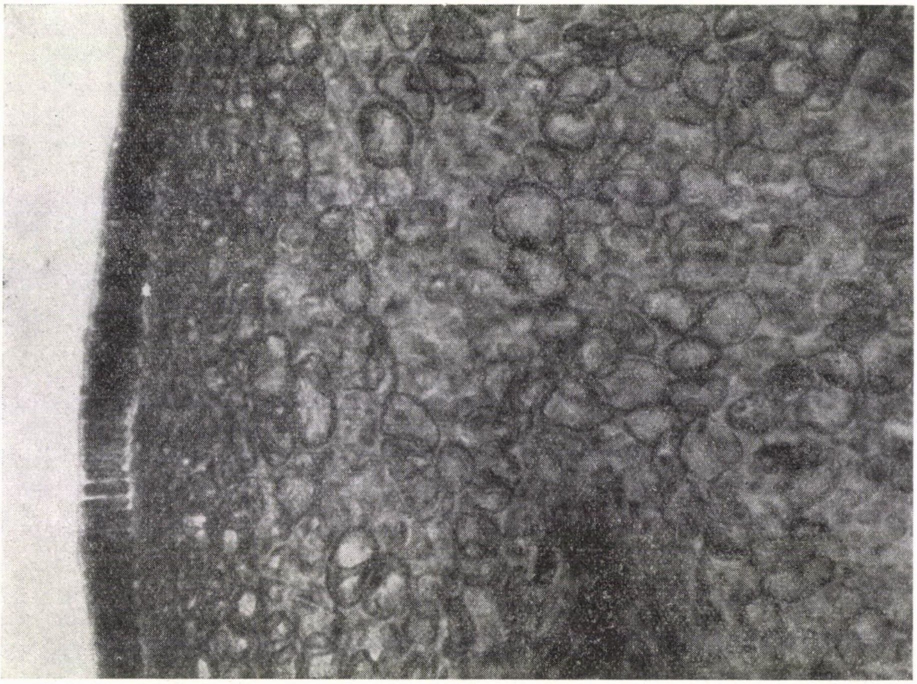

Fig. 3. Outer tissue zone of the developing young fruit wall (obj. 20×, oc. 4×)

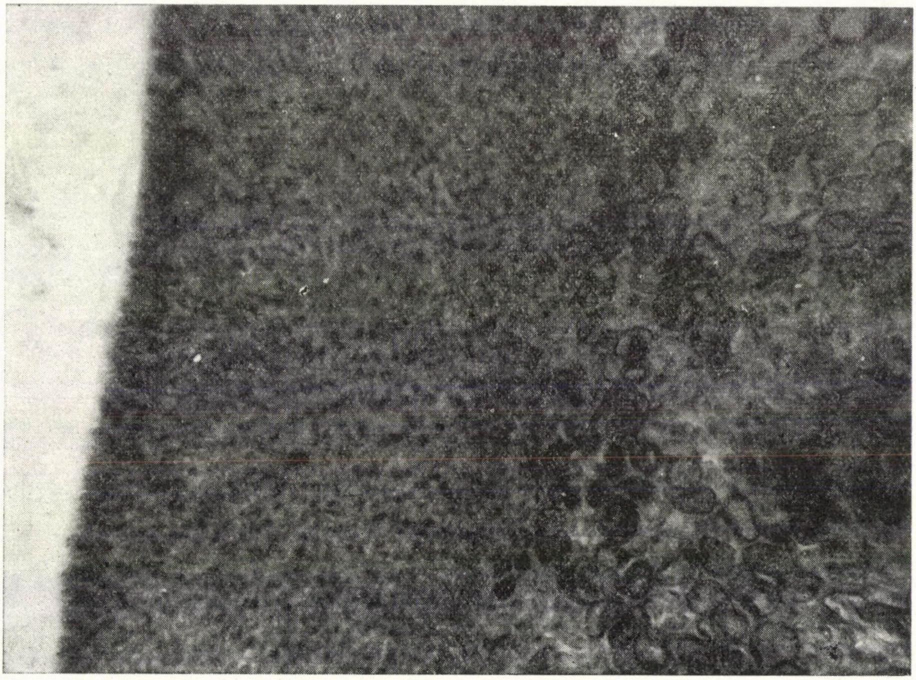

Fig. 4. Inner tissue zone of the developing young fruit wall (obj. 20×, oc. 4×)

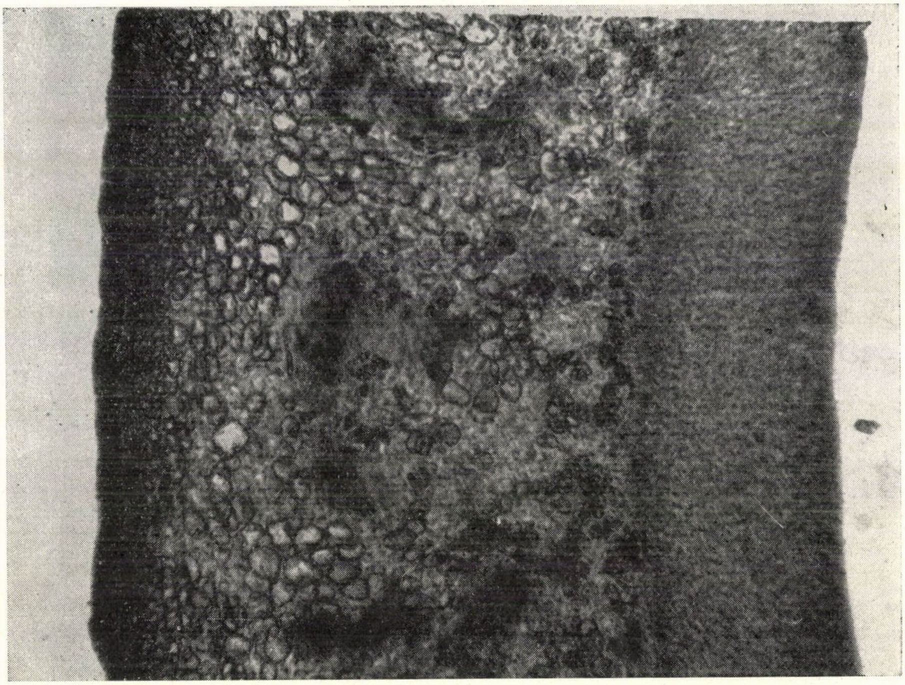

Fig. 5. Longitudinal section of the wall in a somewhat older fruit (obj. 10×, oc. 4×)

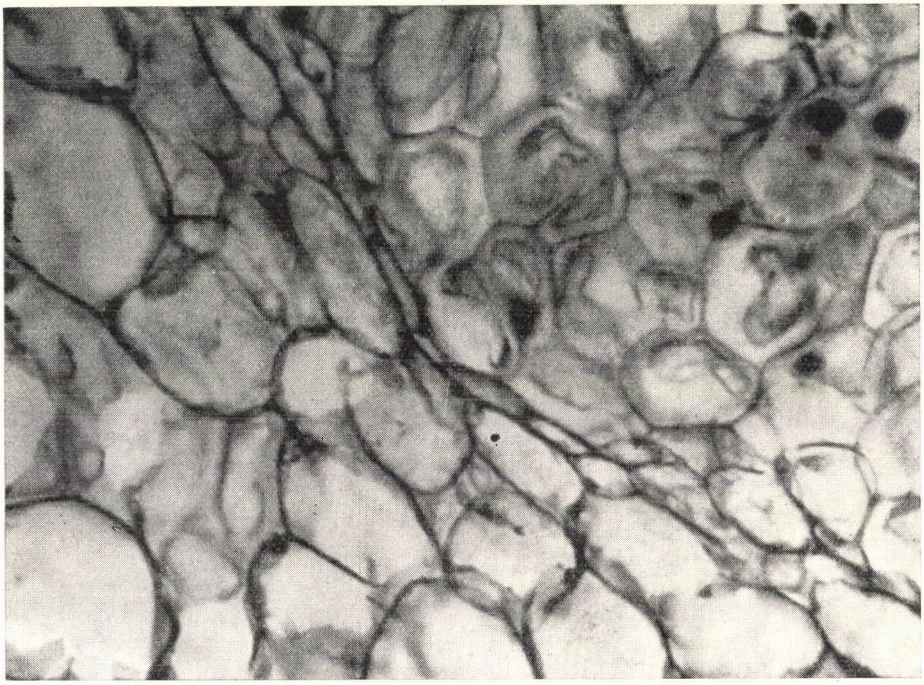

Fig. 6. Layer consisting of transversally dividing cells at the border of the mesocarp and endocarp (obj. 40×, oc. 4×)

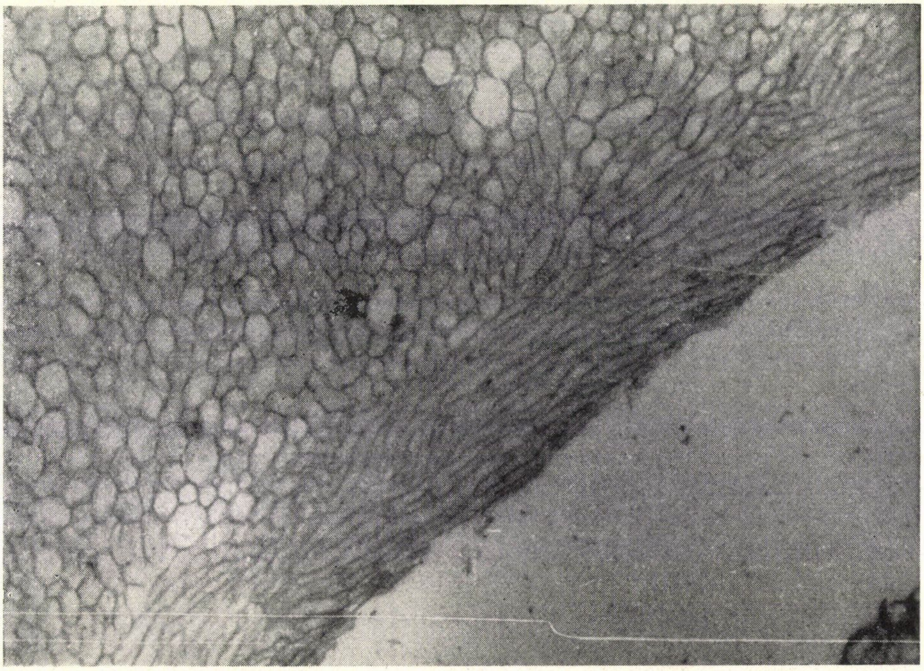

Fig. 7. Lignification of the endocarp has started; the inner layer consists of elongated, the outer one of isodiametric cells (obj. 20×, oc. 4×)

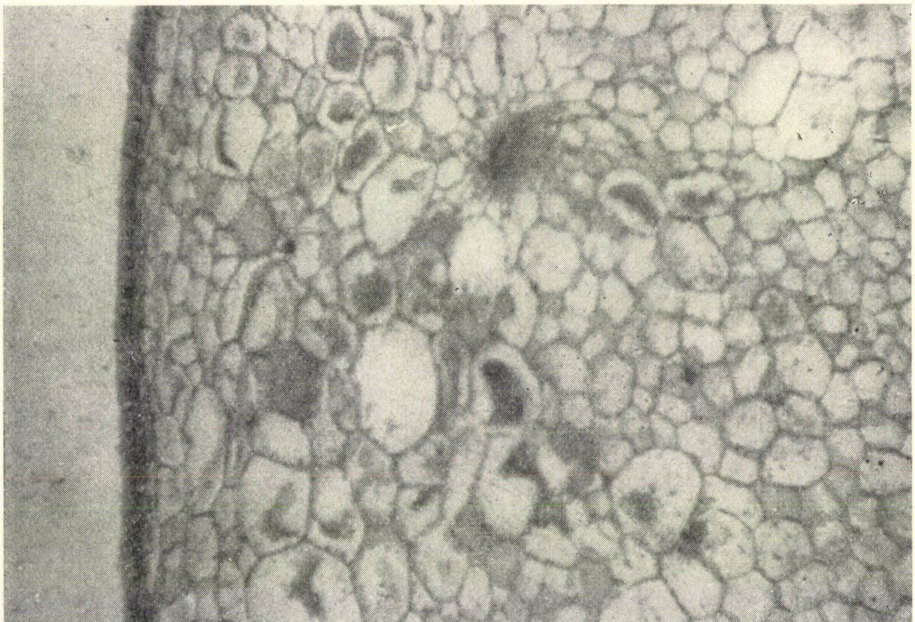

Fig. 8. Detail of ripe fruit flesh with the giant parenchyma cells of the mesocarp (obj. 20×, oc. 4×)

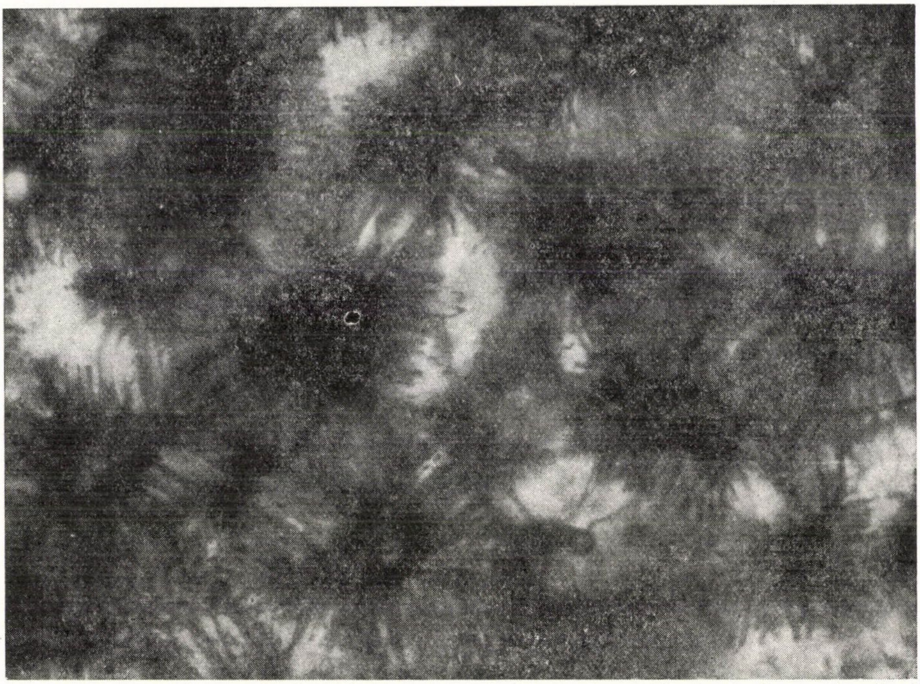

Fig. 9. In a thin section prepared from the endocarp of a ripe fruit the isodiametric sclereids of the outer endocarpic layer appear (obj. 40 ×, oc. 4 ×)

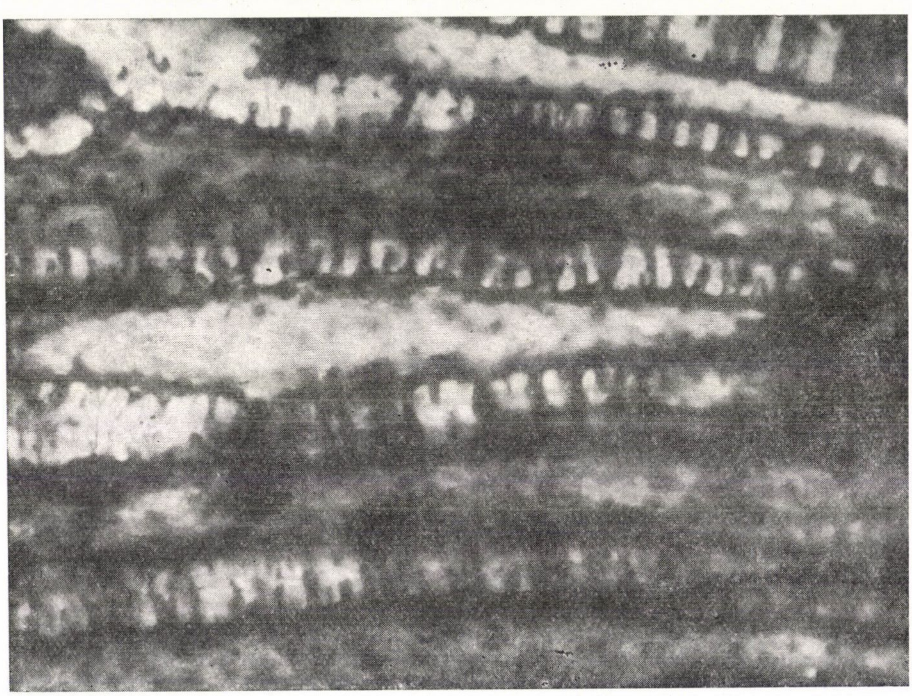

Fig. 10. Elongated sclereids in the inner endocarpic layer (obj. 40 ×, oc. 4 ×)

begins, which advancing outwards spreads over to other cell layers. too. It is in this way that the endocarp develops from the inner tissue zone of the mesophyllum of the fruit-wall and from the inner epidermis. Hairs extending into the fruit cavity are destroyed at that time as a result of cell-wall thickening.

The developing endocarp continues growing to some extent. Namely at the border of the mesocarp and endocarp the innermost mesocarp cells become dilated under the influence of a pressure resulting from the expansion of the endocarp, while a plasma accumulation occurs. Then a repeated cell division takes place with radial walls whereby 4—5 cells are produced tangentially. The new cells grow to some extent, become isodiametric and increase the tissue of the endocarp (Fig. 6). A meristemic activity of this kind is maintained for a while.

At the fourth stage of development the fruit is about 14 × 11 mm large, that is, it has reached about half of its final size. The process of vacuolization goes on especially in the tissue of the mesocarp. Under the epicarp the lamellar collenchyma has already completely developed. Farther in the cells are still of greenish yellow colour due partly to the cell-sap, partly to the chloroplasts the disorganization of which now begins. The mesocarp consists of 30—38 cell-rows, this will no longer change.

The endocarp becomes more and more separated from the mesocarp, and the flattened cells at the border of the two tissue zones no longer divide. Differentiation in the tissue zone has advanced, the cell-walls show bordered pits, but through the pits there is still a plasmatic connection between the adjacent cells. Cells of 10—12 cell-rows in the tissue part next to the fruit cavity become in the meantime highly elongated (Fig. 7). At the place where the edges of the carpel join the cells are generally smaller, flattened and have thin walls.

At the fifth stage the fruit has reached its full size: 20—22 mm width and 24—26 mm length. The cells of the epicarp have elongated parallel with the surface. The cells of the mesocarp are large, the plasma is a thin lining along the cell-wall, the cell-sap in the large central vacuole contains a red colouring matter (Fig. 8).

In the endocarpic cells the plasma has been completely absorbed, the secondary thickening of the cell-walls has continued and the pits have deepened into ducts. The sclereid structure thus developed can be analysed only in thin sections. Owing to pressure conditions prevailing during lignification the shape of cells in the outer layer of the endocarp (putamen) slightly differs from the isometrical and is rather varied. In the inner zone of the endocarp the cells are elongated, fibre-like. These two layers of the endocarp are definitely separated from one another. The final thickness of the endocarp is 55—60 cell-rows (Figs. 9 and 10).

A summarization of the results concerning the fruit organization of cherry has revealed that in the pistil the two parts of the fruit-wall: mesocarp and endocarp are determined from the beginning, although this only becomes obvious at the third stage of development. From the outer part of the mesophyllum the mesocarp, while from the inner meristemic tissue zone and inner epidermis the endocarp develop. The mesocarp shows but a slight growth during its development, while the endocarp increases the number of its cell-rows 6—7 times compared to their original number. This intensive growth is caused, on one hand, by the vigorous division of the endocarpic cells, while on the other hand, the cell division and sclerenchymatization of the mesocarpic cells at the border of the endocarp and mesocarp contribute to this process as well.

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PERICARP THICKNESS IN OPAQUE-2 MAIZE (ZEA MAYS L.) AND ITS NORMAL ANALOGUE

It is a general experience of maize breeders that opaque-2 maize usually matures somewhat later than normal maize (Lambert *et al.* 1969, Paez *et al.* 1969, Kovács *et al.* 1970, 1971). It was shown by Lambert *et al.* (1969) that the opaque-2 maize had nearly 4 per cent more grain moisture during the early harvest period than its normal analogue. Purdy—Crane (1967) observed in slow and fast drying maize hybrids that the differential rates of water loss were due to the physical structure of the pericarp and not to metabolic processes within the kernel. It was observed by Helm *et al.* (1970) that some of the endosperm mutants modify pericarp thickness in maize.

The work reported herein was designed to study if there was any anatomical modification, either in the structure or the thickness of the pericarp of the opaque-2 maize caryopsis, through which the grain moisture must be released during the maturity process.

Incorporation of the opaque-2 gene from the same initial source to several of the inbred lines is one of the current programmes of the institute. Two inbred lines N6 and R61 were crossed with W64Ao_2, the donor of the o_2 gene, and back crossed three times with the normal. The homozygous o_2 forms of these lines and their normal analogues were used in this analysis. Ears obtained from the sib-pollination of single cross hybrids produced from a cross between (N6 $o_2 \times$ R61 o_2) on the one hand, and (N6 × R61) on the other were the materials used in this study. The ears were harvested 56 days after pollination. Ten kernels from each hybrid, five from the upper half of the ear and five from the base of the ear were examined for pericarp thickness with the procedure described below.

Table 1

Pericarp thickness of opaque-2 and normal maize kernels

Material	Kernel placement on the ear	Kernel No.	Pericarp thickness in microns		
			Germinal side	Abgerminal side	Average
Opaque-2	Upper	1.	101.1	157.5	129.3
		2.	141.2	119.3	130.2
		3.	126.4	122.5	124.4
		4.	172.0	173.0	172.5
		5.	154.9	158.3	156.6
		Average	139.1	146.1	142.6
-do-	Lower	1.	113.8	134.3	124.1
		2.	138.3	158.1	148.2
		3.	162.2	123.0	142.6
		4.	137.5	144.0	140.8
		5.	150.6	126.3	138.4
		Average	140.5	137.1	138.8
Average over opaque types			139.8	141.6	140.7
Normal	Upper	1.	107.0	98.2	102.6
		2.	89.0	103.1	96.1
		3.	90.1	123.7	106.9
		4.	113.2	90.5	101.8
		5.	109.8	113.6	111.7
		Average	101.8	105.8	103.8
-do-	Lower	1.	94.2	115.7	105.0
		2.	114.2	113.0	113.6
		3.	99.0	104.2	101.6
		4.	108.1	88.4	98.2
		5.	102.4	87.4	94.9
		Average	103.6	101.7	102.7
Average over normal types			102.7	103.8	103.2

Pericarp strips were removed from kernels steeped in water for 3—4 hours as per the procedure adapted by Wolf *et al.* (1969). Transverse sections, 25 μ in thickness, were cut from the removed pericarp strips both from the germinal and abgerminal sides of the kernel with the help of a handmicrotome. The sections were stained for a short time in diluted UNNA type polychrome methylene blue. The stain was prepared by dissolving 1 gram methylene blue and 1 gram potassium carbonate in 100 ml distilled water plus 20 ml 96% ethanol. The solution was slowly evaporated on a water-bath to 100 ml and filtered (Schneider 1922). The stained sections were mounted in 1 : 1 glycerine water solution. Three measurements on pericarp thickness from each of the two, germinal and abgerminal, surfaces of the kernel, that is six measurements suggested by HELM—ZUBER (1969) were made on each kernel.

Trials were also made to study externally the constitution of the cells of the outermost layer covering the caryopsis. Replicas were made onto nail-polish and examined microscopically. These cells were also examined by adapting the procedure described by UJHELYI (1954) for analysing the anatomy of the epidermis in the *Gramineae* family, which was found to be not too successful for our purpose.

The data obtained on pericarp thickness was analysed statistically using the standard analysis of variance technique (STEEL—TORRIE 1960).

Observations made on the thickness of the removed pericarp strips from the germinal and abgerminal sides of opaque-2 and normal kernels obtained from an upper and a lower placement on the ear are presented in Table 1. Each observation is an average of three measurements made on each side of the kernel. A statistical analysis of these observations is presented in Table 2. It will be seen from this table that the type of the kernel was a major factor in contributing to the observed variation in pericarp thickness, and that the difference between opaque and normal kernels was highly significant. It would also be interesting to note that the replications, the germinal or abgerminal side of the kernel, the placement of the kernel on the ear and various interaction factors were all nonsignificant in contributing to the observed variation. It may be pointed out that the opaque-2 kernels showed on an average a pericarp thickness of 140.7 μ in contrast to only 103.2 μ observed in the normals, that is, it demonstrates an increase of 36.3 percent in pericarp thickness in the presence of the gene o_2 in the recessive homozygous condition over its normal counterpart. The difference is mainly attributable to the effect of the gene, and not to the background, as the hybrids used theoretically had 93.75 per cent genetic background in common. The difference in thickness of the pericarp of opaque-2 and normal kernels is also shown in photographs (Fig. 1).

Table 2

Analysis of variance of data in Table 1

Source	df	Mean squares
Replications	4	214.7
Treatments	7	2045.8**
Side of the kernel	1	21.1
Type of the kernel	1	14044.5**
Placement of the kernel	1	61.7
Side × type	1	1.5
Side × placement	1	163.5
Type × placement	1	17.7
Side × type × placement	1	10.8
Error	28	308.2
Total	39	

** significant at 1% level.

Preliminary studies on the constituent cells of the external layer of the epicarp in opaque-2 and normal kernels have failed to demonstrate any difference. No stomata were observed on either the opaque-2 or the normal maize caryopsis.

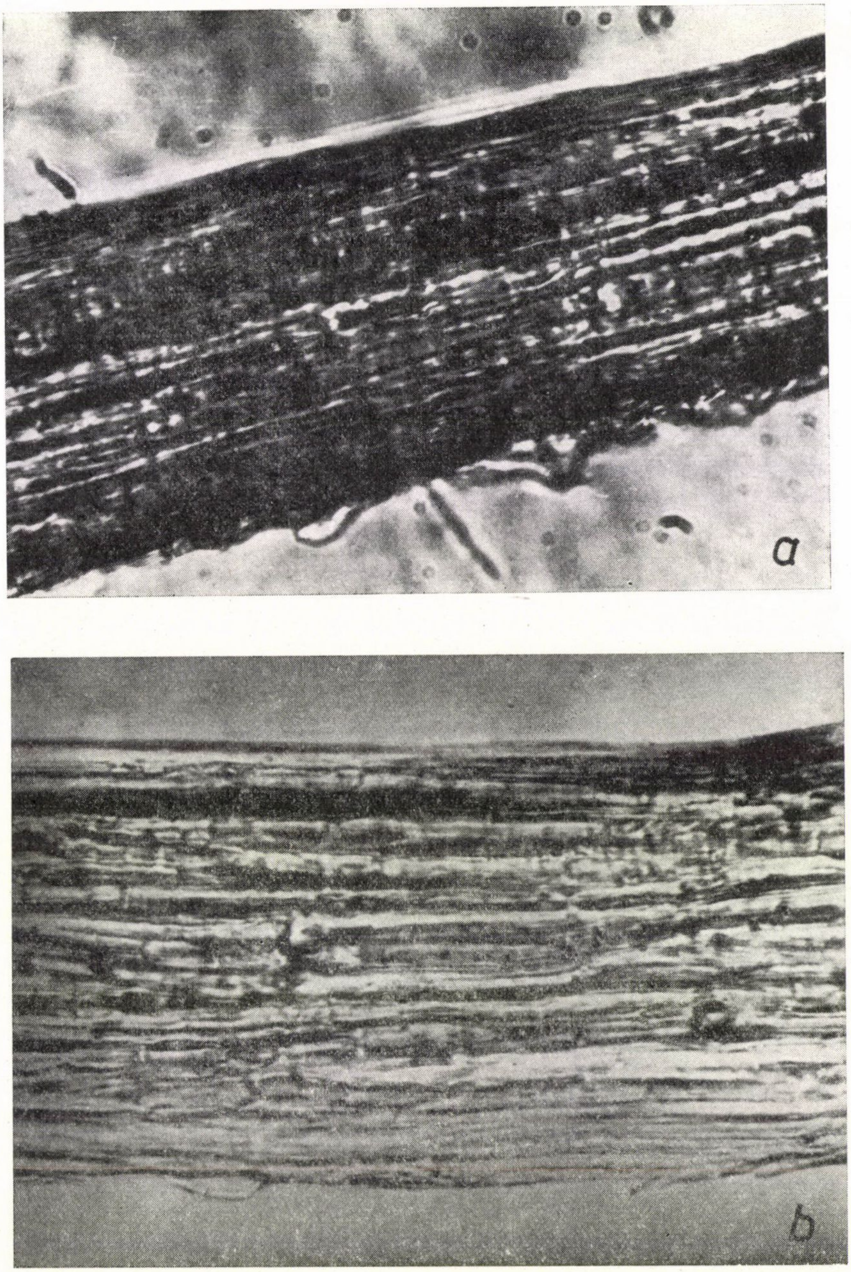

Fig. 1. Sections of the pericarps of *a)* normal and b) opaque-2 maize kernels. Magnification: 500 ×

The drying rate in mature maize has been shown to be directly correlated with the physical structure of the pericarp (Purdy—Crane 1967). The developing endosperm may have a varying amount of compression on the outer tissues which could in turn alter the pericarp thickness (Randolph 1936, Richardson 1960). A lower kernel weight observed in the case of the opaque-2 grains than in the normal (Kovács *et al.* 1971, Alexander *et al.* 1971) could be taken as a measure of the less developed endosperm in the recessive opaque-2 homozygotes. The thicker pericarp thus may be a consequence of the only partially developed endosperm in the opaque-2 kernels which could in turn obviously hinder in their rate of moisture loss.

It may be added that in a yield trial conducted during the year 1972 at Martonvásár comprising of various entries, the normal and opaque-2 (N6 × R61) maize hybrids, the ones in question here, have shown an average of 73.67 and 73.50 days for 50% silking, respectively (Gupta—Kovács unpublished). These data are averages based on 3 replications consisting of observations on 30 plants each. There is hardly, if any, difference between the two types, which could in view of the authors need statistical proof of their being equal, at least based on this trial, in the rate of their development. A similar tendency is to be observed in the data presented by Váczi (1972) which show that essentially the time of silking and tasselling is the same in (WF9 × N6), a normal single cross, and Mv Syn A × B, an opaque varietal hybrid. On the other hand these two hybrids show 30.4% and 35.0% grain moisture at harvest, respectively, demonstrating a difference of 15.1% taking the normal as 100. These data are averages of trials conducted at 7 experimental stations in a national yield trial. Thus, if the rate of development of the opaque-2 and normal maize hybrids is the same till flowering, but the difference in maturity follows later ,the less dry matter percentage generally observed in o_2 kernels than in the normals could be explained as a consequence of the reduced moisture loss during maturity caused by a thicker pericarp.

The absence of stomata from the maize caryopsis is not a surprising observation. They are not present in case of wheat too (Stieber 1962, 1963). They do, however, appear in the apical zone in the case of barley (Stieber 1963).

The present studies made on the pericarp thickness of kernels obtained from opaque-2 and normal maize hybrids have thus shown 36.3 per cent thicker pericarp for opaque-2 maize than the normal. The procedure developed in studying the pericarp thickness was to cut sections from pericarp strips removed from soaked kernels. No stomata were observed on the maize caryopsis either in the case of opaque-2 kernels or in the normals. The thicker pericarp observed in opaque-2 kernels does provide an answer to their cause of having reduced dry matter percentage and relatively more moisture at harvest.

Prepared at the Agricultural Research Institute of the Hungarian Academy of Sciences at Martonvásár.

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THE HEAT BALANCE OF ALFALFA AS RELATED TO ITS IRRIGATION WATER REQUIREMENT

To establish irrigation farming from the agrometeorological point of view, investigations have been carried on at the Agrometeorological Research Station of Szarvas since 1963. The soil of the experimental area is meadow clay. Alfalfa, as a native plant in the district of Szarvas proved to be an ideal indicator in studying the optimum (OE) and actual evapotranspiration (AE) necessary for establishing the irrigation water requirement (IWR), and in measuring these components directly or determining them indirectly, by means of calculation formulas.

In the first five years of the period between 1963 and 1970 the experimental plot met the requirements of a homogeneous surface necessary for applying the turbulent diffusion methods. Besides the heat balance measurements regular observations were made of the temperature, humidity and wind profile. From these data the hourly, daily, five-day, ten-day and monthly values of the actual evapotranspiration were determined with the Bowen ratio and the Monin—Obuhov (1954) turbulent diffusion method. The third method used for determining the evapotranspiration was an empiric formula elaborated by Antal (1968) on the basis of the investigations at Szarvas, which makes it possible to calculate evapotranspiration from data of the meteorological network.

Since on the experimental area direct measurements of the actual evapotranspiration could not be made, the heat balance of the active surface, the energy coverage of the process of evaporation was taken into consideration as a control of the results obtained with various methods.

Direct measuring of the optimum evapotranspiration, or water requirement was carried out by means of Thornthwaite-Mather type evapotranspirometers. Simultaneously with

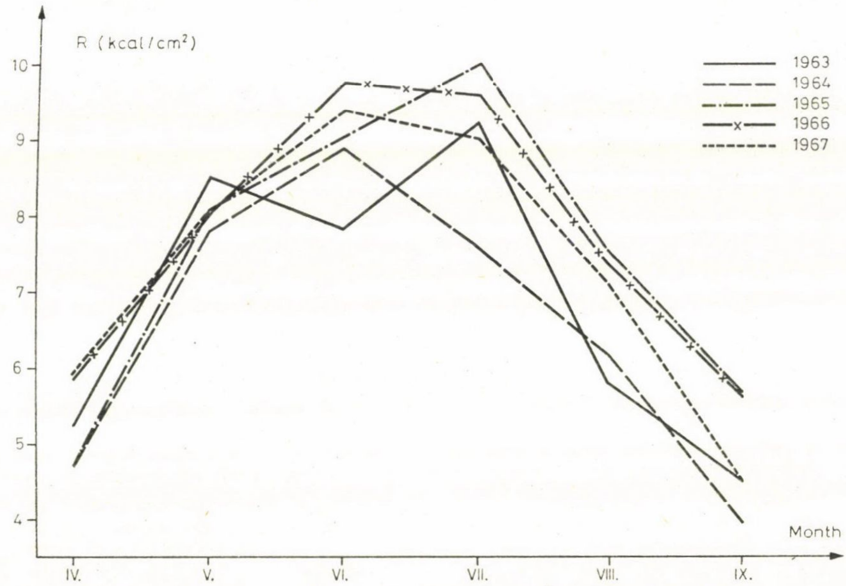

Fig. 1. **Changes in the net radiation during the growing season. (R = net radiation)**

these measurements meteorological, phenological and phenometric observations were also carried out which rendered it possible to calculate the optimum evapotranspiration (OE) from the climatological data.

From the well-known heat balance equation of the homogeneous active surface:

$$R + LE + H + S = 0 \tag{1}$$

the components of net radiation (R) were determined for each hour during the growing season (April—September); short wave global- and reflex radiations were registered, the other (long-wave) components were calculated from the recorded meteorological data. Fig. 1 shows the net radiation during the growing season for each year between 1963 and 1967, while Table 1 presents numerical data of the components for each month and on the average of the five years. Heat amounts discharged by the surface have a negative sign while those taken in have a positive sign.

Apart from the first experimental year with irregular weather conditions the maximum net radiation was always recorded in June or July. As compared to the five-year-average there were about 10—15 per cent differences between the individual years in the monthly values of net radiation. According to our measurements and calculations the net radiation of alfalfa amounted to 50—55 per cent of the global radiation while the albedo showed hardly any change during the growing season and its value ranged between 19 and 22 per cent (WEINGARTNER 1966).

In Table 1 evapotranspiration for the component of latent heat flux was determined by the above mentioned turbulent diffusion method. The calculation was described in detail in an earlier paper (TÓTH 1966). As it can be seen from the data the energy used for evapotranspiration was 66 per cent of the net radiation on an average while in years with sufficient water supply (e.g. in 1965) it amounted to even 73 per cent of the net radiation.

In the first two-thirds of the growing period the soil heat flux extracted heat from the active surface, while by August the upward flow of heat replaced the energy losses of the active surface.

Table 1

The values of the heat balance components in different months and growing seasons

Year	IV	V	VI	VII	VIII	IX	IV–IX
Net radiation							
1963	5243	8499	7838	9192	5772	4476	41020 (cal)
1964	4519	7785	8893	7569	6143	3937	38846
1965	4680	8099	9020	9999	7505	5657	44960
1966	5834	8013	9749	9571	7335	5616	46119
1967	5939	8085	9388	9000	7072	4457	43941
Mean	5243	8096	8978	9066	6765	4829	42977
Latent heat flux							
1963	—4017	—5104	—4796	—4928	—4481	—2693	—26019 (cal)
1964	—3791	—4001	—4021	—4210	—5761	—2965	—24749
1965	—4320	—4980	—7009	—7356	—4825	—4690	—33180
1966	—3557	—5767	—4974	—5862	—4620	—4114	—28894
1967	—4398	—6431	—5094	—4245	—4865	—4107	—29140
Mean	—4017	—5257	—5179	—5320	—4910	—3714	—28397
Sensible heat flux							
1963	— 975	—3249	—2646	—4431	—1417	—2060	—14778 (cal)
1964	— 648	—3419	—4755	—3322	— 528	—1125	—13797
1965	— 143	—2586	—1634	—2557	—2805	— 985	—10710
1966	—1736	—2292	—4587	—3532	—2787	—1615	—16549
1967	—1374	—1294	—3970	—4593	—2330	— 458	—14019
Mean	— 975	—2568	—3518	—3687	—1973	—1249	—13970
Soil heat flux							
1963	— 251	— 146	— 396	167	126	277	— 223 (cal)
1964	— 80	— 365	— 117	— 37	146	153	— 300
1965	— 217	— 533	— 377	— 86	126	18	— 1070
1966	— 541	46	— 188	— 177	72	112	— 676
1967	— 167	— 360	— 324	— 162	123	108	— 782
Mean	— 251	— 272	— 280	— 59	118	134	— 610

The sensible heat flux was calculated from the heat balance equation (1) as a residual term. Its value changed depending on the methods used for determining the evapotranspiration.

The average courses of the heat balance components in the growing season are illustrated in Fig. 2 taking into account the actual evapotranspiration determined by the turbulent diffusion method (part a) as well as that calculated with the mentioned empirical formula (part b).

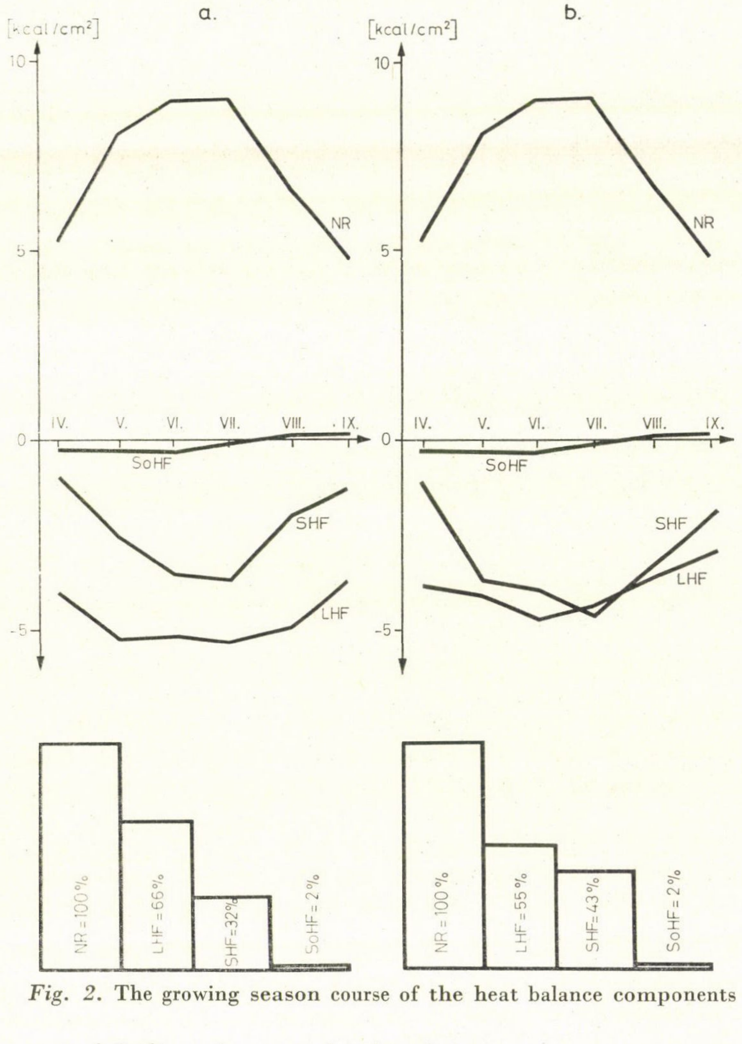

Fig. 2. The growing season course of the heat balance components

In the lower part of the figure the sums of the heat balance components are expressed as a percentage of the net radiation. The values of the evapotranspiration obtained using the turbulent diffusion method are always higher not only for the growing season but — as it is seen in Table 2 — for most of the five-day-period too. In the whole growing season this difference was 70 mm on an average, but in more rainy years, months or five-day-periods it showed a decreasing tendency.

A comparison of the methods selected for the determination of the actual evapotranspiration is demonstrated on the example of the year 1965; the results obtained with the heat balance method, turbulent diffusion method and empiric formula respectively, are presented in Table 3.

The first two methods gave nearly identical results for the growing season: about 550 mm actual evapotranspiration compared to the 490 mm obtained with the empiric formula. Comparing the methods on the basis of the five-day-period values for the growing seasons too, we find that the difference between the results obtained with the empiric formula on one hand,

Table 2

Five-day-values of evapotranspiration calculated with the turbulent and empiric method respectively

Time		$AE_{turb.}$	$AE_{emp.}$	$AE_{turb.}$—$AE_{emp.}$	
April	1	8.7	9.9	—1.2	2.6 (mm)
	2	8.3	11.2	—2.9	
	3	9.6	9.5	0.1	
	4	13.6	11.9	1.7	
	5	13.5	10.1	3.4	
	6	13.2	11.7	1.5	
May	1	18.2	11.1	7.1	19.2 (mm)
	2	13.4	10.9	2.5	
	3	14.6	12.2	2.4	
	4	13.7	12.7	1.0	
	5	13.0	8.9	4.1	
	6	15.4	13.3	2.1	
June	1	17.8	13.7	4.1	5.3 (mm)
	2	15.2	11.6	3.6	
	3	13.5	11.6	1.9	
	4	12.5	12.3	0.2	
	5	14.3	15.3	—1.0	
	6	11.9	15.4	—3.5	
July	1	11.6	12.3	—0.7	15.9 (mm)
	2	13.3	12.1	1.2	
	3	17.1	12.1	5.0	
	4	14.7	11.2	3.5	
	5	16.1	12.0	4.1	
	6	17.2	14.4	2.8	
August	1	15.0	12.1	2.9	21.6 (mm)
	2	13.0	11.1	1.9	
	3	13.1	8.6	4.5	
	4	12.7	10.3	2.4	
	5	15.3	9.6	5.7	
	6	13.8	9.6	4.2	
Sept.	1	13.5	9.9	3.6	12.2 (mm)
	2	11.8	8.2	3.6	
	3	9.4	7.9	1.5	
	4	8.6	8.4	0.2	
	5	9.1	8.3	0.8	
	6	10.0	7.5	2.5	

Table 3

Actual evapotranspiration of alfalfa as calculated with various methods
1965

Month	$AE_{turb.}$	$AE_{heat.}$ balance	$AE_{emp.}$
April	72	51	54 (mm)
May	84	90	82
June	118	110	97
July	124	128	109
August	81	95	84
Sept.	79	73	68
April—Sept.	558	547	494 (mm)

Table 4

Sums of precipitation and irrigation water requirement

Year	Precipitation	Irrigation water requirement	Total (mm)
1963	239	365	604
1964	186	391	577
1965	421	106	527
1966	283	246	529
1967	282	252	534
1968	360	306	666
1969	292	266	558
1970	375	150	525
Mean	304	260	564

and those obtained by the turbulent diffusion and heat balance methods on the other, relates inversely to the moisture supply. The difference and this kind of relation to the moisture supply can be explained by the following fact. The turbulent diffusion method and the heat balance method are built — as it is known — on physical (energetical) processes taking place above surface, and give the amount of water evaporating from the surface and circulating in the atmosphere near the ground, respectively, irrespective of the soil layer from which the given amount of water may have originated. At the same time, the empirical formula emphasizes the role of the soil moisture content besides the vapour uptake capacity of the air. The difference is obviously caused by the fact that in our calculations we do not take into account the whole soil layer from which the crop extracts the water.

Namely, in order to be able to extend the procedure in space and time we only took a 1 m soil layer into account in our present studies. This will facilitate our further work, since the soil physical characteristics and the soil moisture data to this depth are available from a number of sites in the country. However, it is known that this deep-rooted fodder plant can take up water even from a three-meter deep soil layer.

If in the following simplified equation of the water balance

$$P + \Delta W + GW = AE + RO \qquad (2)$$

(where P = precipitation, ΔW = changes of water supply in the soil layer, GW = water taken up from the groundwater — in the present case from the soil layer below 1 m —, AE = evapotranspiration and RO = run-off) we substitute the measured values of precipitation and soil water changes, as well as the values of evapotranspiration calculated with the turbulent diffusion or heat balance method, and under lowland conditions neglect the run-off, then — taking as a basis — the five-year-average

$$GW = 65 \text{ mm},$$

that is, from spring to autumn water intake was increased from the moisture content of the soil layer below 1 m by about 65 mm. In districts with more precipitation where the root system of the crop is not forced to penetrate into deeper layers to obtain water, further, with all those crops whose root systems are found in the upper 1 m layer, this GW component does not cause any problem.

Thus, if in our present investigations, not having actually measured the values of evapotranspiration we accept the results obtained by the heat balance or turbulent diffusion method as "etalon" data, further, assume that formula (2) describes the water balance of the experimental area correctly, then the 70 mm difference between the various calculation methods is caused by all means by the moisture content of the soil layer below 1 m left out of consideration.

Knowing, however, that when measuring the amount of water applied through irrigation systems a mistake of ±20 mm can easily be made on one occasion, under Hungarian conditions we consider it sufficient to reckon with a 1 m soil layer when determining the irrigation norm and planning the water management, even in the case of an alfalfa crop. Thus, actual evapotranspiration in the period of 1963—1970, as well as that between 1901 and 1950, in the period used for determining distribution in space, were calculated by the means of ANTAL's (1968) empirical formula.

In Fig. 3 water need, actual evapotranspiration, irrigation water requirement and precipitation during the growing season are shown for the years 1963—1970. According to our measurements and calculations, the irrigation water requirement of the alfalfa was 260 mm in the average of these eight years — as the optimum evapotranspiration was 690 mm and the actual one 430 mm; and if we take into consideration that an average of 50 mm irrigation water was applied in the growing season on the experimental plot in order to moderate the difference in development between the plants in the evapotranspirometer and the environment, then the irrigation water requirement of the alfalfa can be determined as 310 mm in the district of Szarvas. According to investigations of KOVÁCS (1968) the irrigation water requirement of alfalfa is 280—340 mm in the Trans-Tisza- and Danube—Tisza Midregion.

The crop needs more than half of its irrigation water requirement (55 per cent) in July—August, and 70 per cent of the irrigating water ought to be supplied in the June—August period.

The irrigation water requirement given above — being an average — may range between wide limits. Namely, water content remaining in the soil from winter precipitation, rain falling in the growing season as well as the trend of temperature may modify the amount of irrigation water required to a considerable extent.

The time to irrigate the alfalfa crop is usually connected with the time of cutting, especially in the case of sprinkling irrigation. In the Hungarian practice hay is harvested three or four times a year, and irrigation is applied subsequently irrespective of the weather conditions. After the first and fourth cutting the amount of irrigation water is less than when supplied in the middle of the growing season, with water reserves usually existing in the soil at the beginning of the season, and mid-summer peaks of water consumption shown in Fig. 3 taken in con-

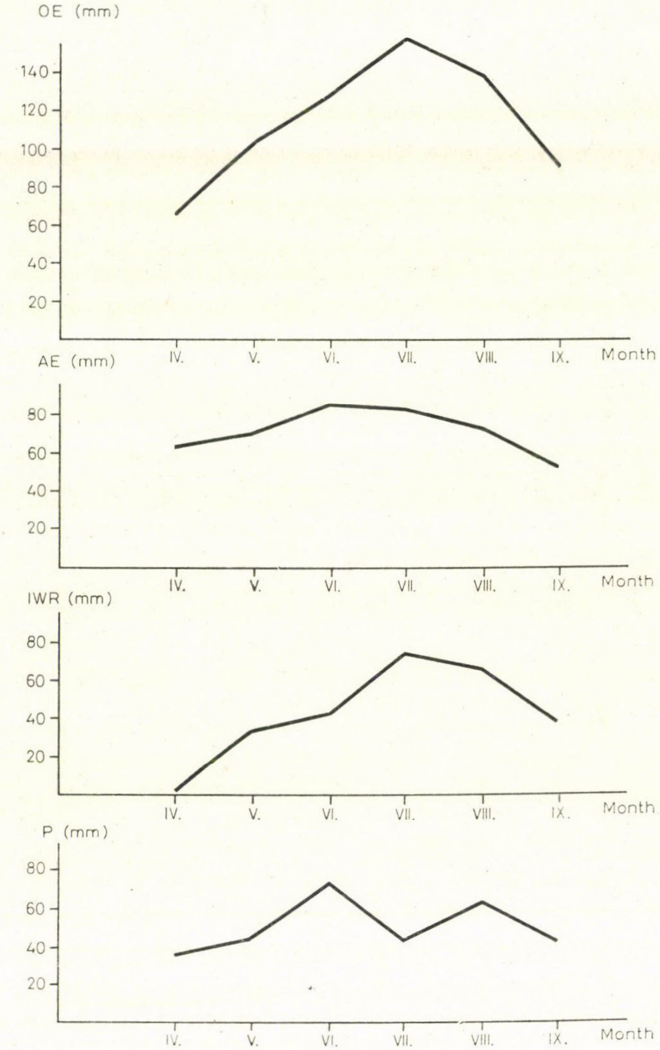

Fig. 3. The optimum evapotranspiration (OE), actual evapotranspiration (AE), irrigation water requirement (IWR) and precipitation (P) in the growing season, on the average of the years 1963—1970

sideration. Thus, on our experimental area the 310 mm water can be distributed over the occasions of cutting in the following way: after the first cutting 70, after the second 90, after the third 90 and after the fourth cutting 60 mm irrigation water should be applied.

The water need of alfalfa in the cuttings from April to September 1965 is shown by the saw-curve of Fig. 4. The optimum evapotranspiration accumulated from the five-day values increased in each successive cutting, but in the last three five-day periods of the growing season (April—September) following the fourth cutting water consumption amounted to only 60 per cent of that in the three five-day-periods following the third cutting.

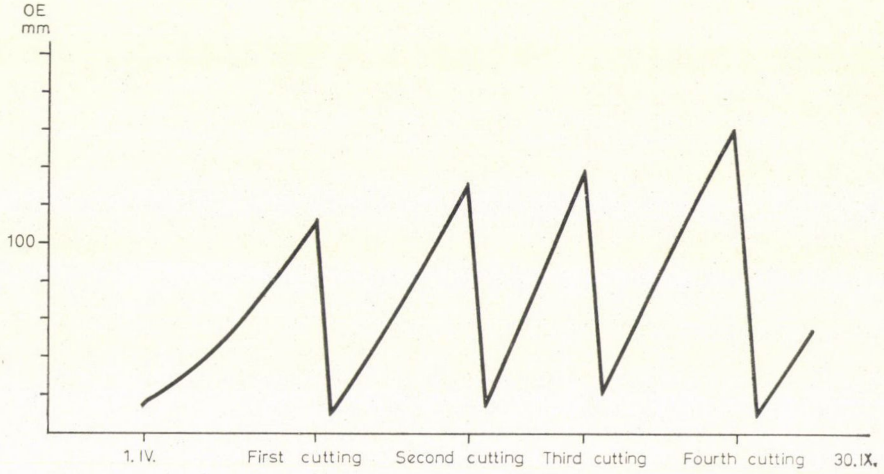

Fig. 4. **Accumulated amounts of water requirement per cutting**

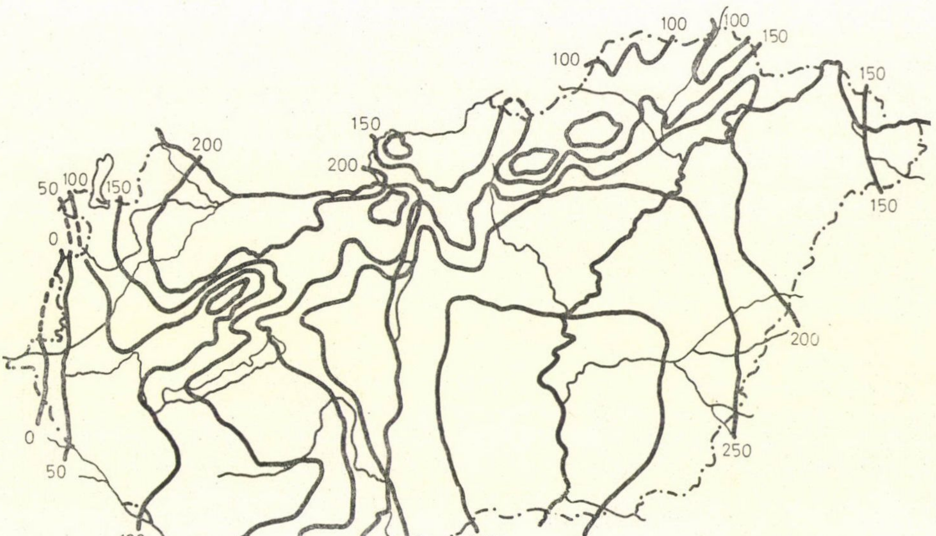

Fig. 5. Irrigation water requirement of alfalfa in Hungary (1901—1950), (April—September

The amount of precipitation and the calculated irrigation water requirement in each growing season exceeded 500 mm according to the data in Table 4. Otherwise, as it is found in the agricultural literature (KOVÁCS 1966) this amount of water ensures high yields of hay.

With an optimum water supply the average yield of alfalfa hay was 157 q/ha, a considerable yield increase compared to the yield of about 35 q/ha obtained without irrigation. To produce 1 kg dry matter the alfalfa as a crop of high water need used 496 kg of water according to our measurements.

The distribution of the irrigation water requirement for lucerne can be seen in Fig. 5. Fifty-year-averages (1901—1950) of climatological data from 54 meteorological stations were used for the calculations (Posza—Tóth, 1969). According to the climatological data the irrigation water requirement of alfalfa ranges between 0 and 300 mm in Hungary in the average of this long period. We can neglect the far west part of Hungary where the amount of the yearly irrigation water is less than 100 mm. The norm of the irrigation water is at least 300 mm or more in the Great- and Little Plain (the central and north-western part of Hungary). We have to reckon with about 150 mm water deficit even in the more rainy Transdanubia.

The results of the agrometeorological investigations described in this paper provide information for planning new irrigation systems, water management, as well as for the extension of irrigated fodder production — a question of the day. Beyond this, the methods elaborated during the investigations are suitable for providing concrete irrigation projections, if there is a meteorological station operating in the vicinity of the given area, and the initial water content and the most important physical characteristics of the soil are known at the beginning of the growing season (field capacity, wilting point, volume weight). Thus, for the agricultural practice, we have extended the results of the agrometeorological investigations beyond the basis of the irrigation norms to forecast the time and amount of irrigation. For some years we have been giving advice regularly concerning the irrigation of three or four crops of some large-scale farms. According to the crop yields obtained on these farms, the irrigation carried out at proper times with sufficient amount of water resulted in high yields every year.

Prepared at the Central Institute of Atmosphere Physics, Budapest

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ADVANCES IN THE CONSTRUCTION OF MODERN ELECTRON MICROSCOPES

The present paper reproduces the substantial contents of a lecture held at the Symposium for Electron Microscopy in December, 1972 in the house of the Hungarian Academy of Sciences.

Busch in 1926 proved theoretically that a rotational-symmetrical magnetic or electric field exerts a real lens effect on electron rays. At that time nobody guessed that the basis of electron microscopy had been created by this discovery. It was a long way from the air-core coil of extraordinarily long focal distances energized by a current flow to the magnetic poleshoe lens introducing the epoch of electron microscopy by its short focal length of a few millimeters.

The first commercial magnetic electron microscope, named "Übermicroscope" (Supermicroscope) was produced in 1937/38 by Siemens. The first electrostatic Supermicroscope was delivered 2—3 years later by the AEG Research Institute. Both these basic types of the instrument were constructed and created the starting point of the rapid development, in the years after the war. The resolution of the electron microscope up to the present time is not determined by the wavelength of the electron rays, but exclusively by the projection failures of the electron lenses. The two most significant of these are the chromatic and the spherical faults. The effect of the chromatic fault is that the electrons with different speeds are not focussed in the same point; so this fault demands uniform electron energy for its correction. The effect of the aperture fault is that the rays coming into the lens from an object point in a greater angular incidence relative to the optical axis suffer a greater diffraction than those with a lower angular incidence. For keeping this fault within tolerable limits the electron lenses may be executed to process the rays close to the axis, i.e. the paraxial rays. The spherical fault practically could not be corrected up to now, so it is the one which determines the limit value of the resolution. With the magnetic objectives this theoretical limit lies at about 2 Å, whereas in the case of the electrostatic objectives the aperture fault is higher by an order of magnitude and the limit resolution capacity amounts to about 10 Å.

The first generation electron microscopes were still far from these theoretical resolution limits. The symmetry errors in the rotational symmetrical objective fields produced another fault, namely astigmatism. An astigmatic lens permits no perfect image sharpness, as its focal distances in both normal planes are not accurately equal. This difficulty was only overcome during the fifties, with the introduction of the stigmators, whose function is to compensate the rotation-unsymmetric part of the objective field with the help of an electron-optical lens the intensity and direction of which can be externally adjusted.

The transillumination microscopes of both basic conceptions, i.e. both the electrostatic and the electromagnetic types, have been developed further. Advantages and disadvantages could be referred to in the case of both types. Difficulties with the magnetic type apparatuses arose due to the separate stabilizing of the high voltage and the lens currents. The electrostatic type instruments along with their higher spherical errors had the advantage of their simpler construction and far lower demands for high voltage stabilization and these were reasons in the favour of these types.

The decision between these instrument types has been influenced by the enormously developing electronics. The development of the electronic construction units and circuitry technics facilitated the construction of highly stable Current- and voltage supplies more and more, whereby the electron-optical advantages of the magnetic objectives could be exploited even further.

In due course a new type of the instrument appeared: the magnetic high performance microscope. It is well justified to regard the SIEMENS-Elmiskop I as the first instrument of this class. It doubtlessly influenced the later microscope constructions in a decisive manner. Its electron-optical construction consisting of a two-stage condenser, an objective, an intermediate lens and a projector lens became practically the standard. The novelty in this construction was the double condenser.

For the highest magnifications the object needs very high electron current densities in order to attain a well visible screen image. On the other hand the irradiation energy on the object must be as low as possible to avoid image disturbances due to upcharging and object damages. The only way of meeting both requirements is to limit a high current density over a very limited area of the object. This is done by the double condenser.

Unfortunately the double condenser of the instrument Elmiskp I could not be utilized fully during the first period, because with the reduction of the object irradiation the irradiated object area became more and more contaminated by the atmosphere of the residual gases, heavily impairing the high resolution.

Only in about 1960 could the condensable hydrocarbons of the residual gaseous atmosphere be bound by the introduction of object room cooling, i.e. a cooling trap erected in the surroundings of the object. This object room cooling permitted the utilization of reduced irradiation in order to improve the resolution by the double condenser.

Instruments of ever higher capacity have been put to the disposal of research in the fields of solid state physics, chemistry, medicine and biology. Preparation technics have developed at the same rate giving new impact to the construction of the microscopes in return. The development of the ultramicrotomes in the middle fifties extended the field of application of the electron microscope very greatly. With the appearance of this preparation process immense advances were attained in the various research fields of biology and medicine, the greatest fields of application of electron microscopy today. In tissue sections the attainable resolution is limited by the excision itself and the contrasting processes to about 20 Å. Therefore it was obvious that first of all a microscope of as easy handling as possible had to be developed for this field of application. These "medium class instruments" have today a resolution of about 8—10 Å.

The demands of solid state physicists, especially the metallurgists are fundamentally different. They want first of all high acceleration voltages so as to be able to carry out real investigations of the structural conditions in thicknesses of about 1 μm of the work materials. After the pioneering works of Dupouy in Toulouse Super-voltage electron microscopes with acceleration voltages adjustable between 0.5—3 Mio V were developed in Japan, the United Kingdom and the USA. Such an instrument needs a special hall. The high voltage supply is arranged in a pressure vessel above the column of the microscope. The image is observed through 20—50 cm thick lead-glass windows.

The electron microscopes may be grouped today as follows:

	Resolution	kV
1. Small instruments	$>$ 30 Å	30—50
2. Medium class instruments	$\approx$ 10 Å	$\approx$ 60
3. High capacity instruments	$<$ 5 Å	20—120
4. Supervoltage instruments		$>$500

After this short survey let us examine the development trends of the last years on the example of two special instruments, a medium class and a high capacity instrument of the firm OPTON Feintechnik, Vienna.

The medium class instruments of the type EM 9

Up to about ten years ago the electron microscope was an instrument whose handling necessitated a specialist. With regard to the high importance of electron microscopy in wide fields of the natural sciences and technics the electron microscope became ever more a natural inventory of the scientific laboratories. Consequently an instrument that could be operated easily, not only by the research workers of all the professional fields, but also by the auxiliary staff, ensuring good results was needed.

An instrument operator with no expert technical and physical education generally had problems with

1. the safe handling of the high vacuum pump systems,
2. the adjustment and operation of the microscope,
3. the replacement of the object with the risk of erroneous operation,

4. the exposure unit which had been as primitive in all the microscopes, as the first photo-cameras with the manually operated objective covering caps. The results of the work were endangered just by these expositioning devices, even in the case of experienced electron microscope operators, as the shots of the most beautifully adjusted objects often proved to have been not optimally irradiated.

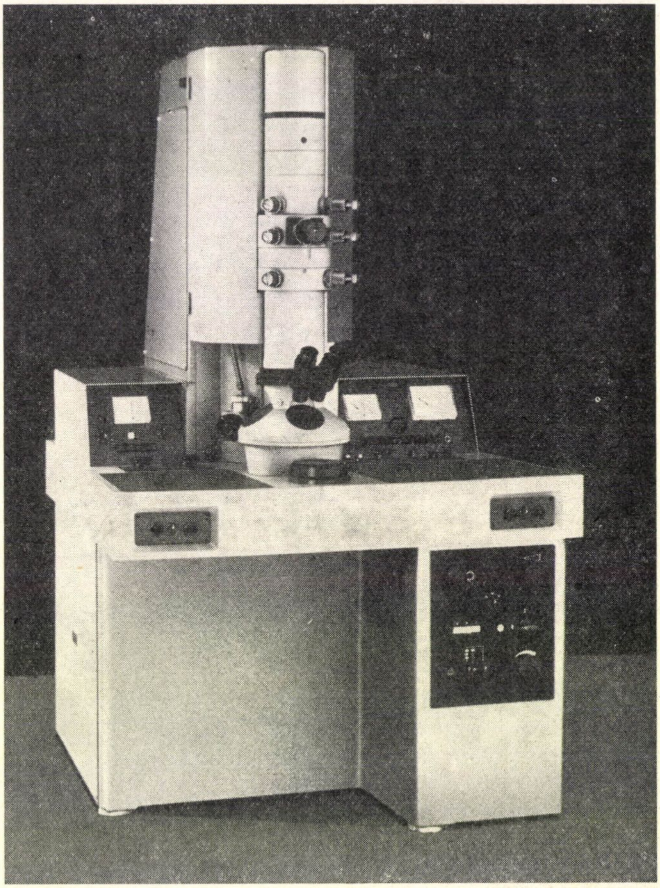

Fig. 1. Medium class electron microscope type EM 9 S-2

The type EM 9 went far in eliminating these difficulties. Fig. 1 shows the general view of the instrument. EM 9 is a compact instrument with all its constructional groups integrated in it. The low number of the control elements is remarkable. The switch and adjustment controls are arranged on the frontplate of the instrument. The few control knobs for operating the instrument in running condition are found in close assembly on the table surface to the right and left of the column.

Switching on is trivial. The main switch is switched on and it pushes down the command key "High vacuum". Everything else is performed by the pumping automatics.

The pump system consisting of a pressure controlled valveblock, an oil diffusion pump and a fore-vacuum pump is run stepwise to the high vacuum pump position. When the minimum

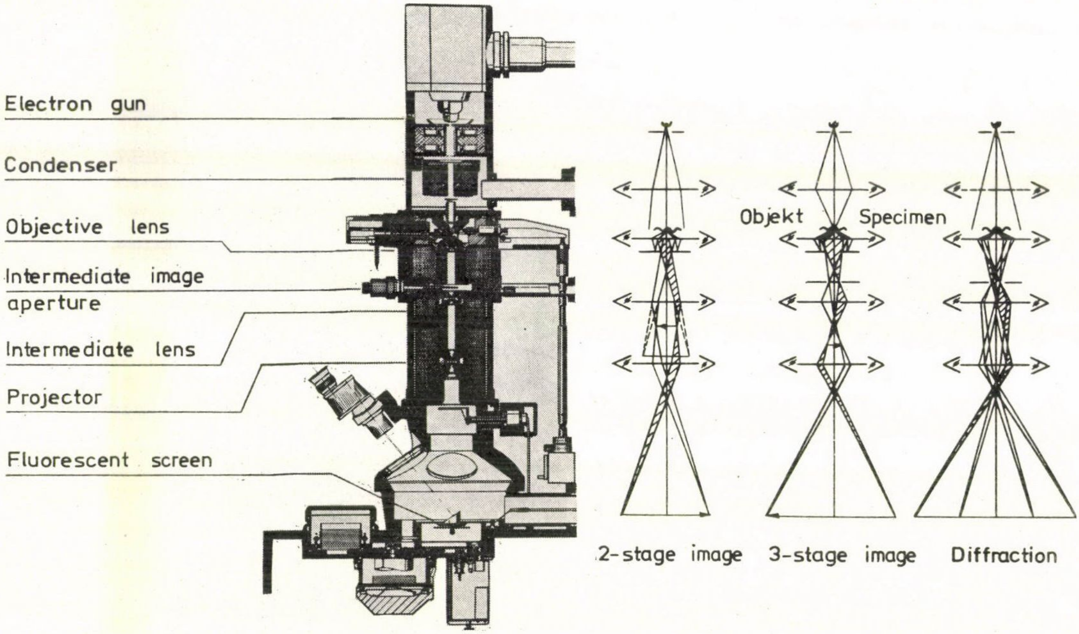

Fig. 2. Section through the column of the instrument EM 9 S-2 with ray paths (2-stage, 3-stage, fine range deflection)

operational vacuum is attained, this condition is indicated on the control desk. High vacuum and cathode heating can be switched on only now.

The airing valve of the column is also secured in a way that it can only be opened, if the high vacuum valve between the column and the diffusion pump is closed.

If an incorrect command is given it is not carried out by the automatics, so this pumpstation is absolutely foolproof, ensured against erronous handling.

Fig. 2 shows a section of the column with the possible ray paths. The column consists of the radiation source, the condenser, the objective, the intermediate lens and the projector.

The radiation source is mechanically fixed on the column. The electron ray radiated by the source is led to the optical axis of the objective by an electromagnetic adjustment system sweeping the beam in two planes. The axes of both the condenser and the intermediate lens can be made to coincide with the axis of the objective by simple shifting. These lens-adjustments, if carried out once, remain unaltered for a long time. The objective is supplied with an electrically centrable stigmator for the correction of any astigmatism. In the rear focal plane of the objective there is an objective aperture diaphragm. Three diaphragms of seven bores each, of various diameters may be exchanged under vacuum.

The adjustments in the routine-like operation of the microscope are limited to the beam adjustment and the compensation of the astigmatism.

Magnifications can be adjusted continuously, without recording between 900—60,000 ×. Small magnifications up to 5,000 × are produced in two stages, i.e. the objective and the intermediate lens together form the first projection stage. The real intermediate image through the projector is projected on the final lighting screen in a further magnification by the projector. Magnifications over 5,000 × are projected in three stages, creating two real intermediate images. The magnification selector switch permits the fast adjustment of five stable, calibrated magni-

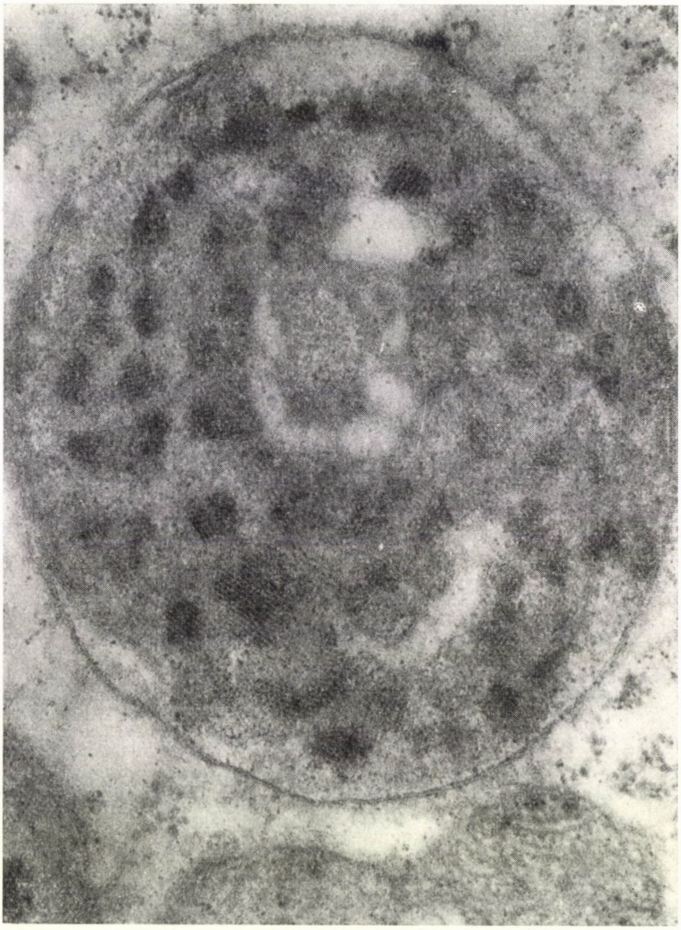

Fig. 3. Yolk grains, Blastula, Limnaea stagnalis L., prep. by: Drs. J. G. Bluemink, Zool Inst. Utrecht University. 72,000×

fication stages, facilitating the routine-like works. When the magnifications are changed, the image centre stays unaltered. Also a surveying magnification of about 150× is available.

The objects are replaced by an absolutely operation-safe eccentric rod-sluice. The object rod is introduced into the sluice, is rotated in a bar link guide and pushed into the instrument, where the object holder seated on the rod point is decoupled and led onto the object table. The sluicing time is less than 10 seconds. The shifting of the object in two directions perpendicular to each other is performed by two easily accessible drives; the table position is indicated by numbers.

The shooting equipment of the instrument EM 9 has been unsurpassed for the last 10 years. It consists of a motorically driven plane film exchanger with a light-optical film-numbering device and the irradiation automatics controlling an electromagnetic lock under the projector.

Fig. 4. Tobacco-mosaic-virus, obliquely steamed by WO_3, prep. by: Max Planck Inst. for Virus Research, Tübingen. 91,000 ×

The electron current density is continuously measured in the image centre by the irradiation automatics. An electrically isolated central area of the fluorescent screen serves as electron capture. This electron current is measured through an amplifier and the measured value is transferred into a storage. Another component of the automatics is the timer. For one shot the fluorescent screen is flapped up. At the start of the screen motion the lock shuts and the storage, which is isolated from the amplifier, stores the last measured irradiation value. If the fluorescent screen is in its upper end position, the lock opens and the irradiation period starts. Simultaneously, the storage, essentially a condenser, is connected to the timer. The timer converts the stored value into a time value and ends the irradiation period by shutting the lock. The end of the irradiation period is optically indicated. Now the fluorescent screen may be shut again. If the screen has returned to its resting position, the lock opens and the observable electron image becomes visible again.

The automatic plane film exchanger transfers the next plane film cassette — ready for shooting, — within 6 seconds under the fluorescent screen. During the shooting a five-digit

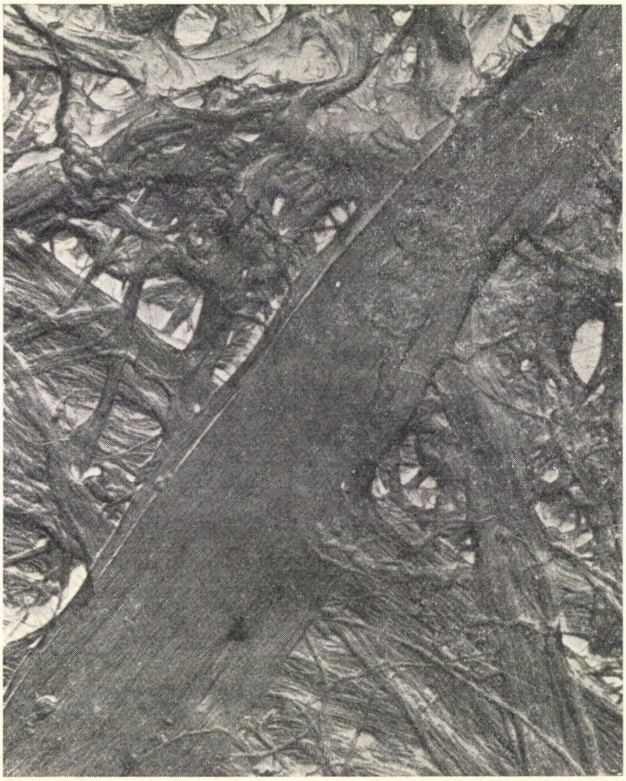

Fig. 5. Cotton linters, Platinum-carbon surface impression, prep. by Dr. Hunger, Inst. for cellulose-chemistry, Darmstadt Technical College. 12,000 ×

number is marked as shot-number on the negative to avoid any accidental interchanges of the negatives.

Naturally the irradiation automatics can be adapted to the sensitivity of the actually used films. The possible irradiation times vary between 0.1—60 seconds.

This detailed description of the medium class instrument is intended to show that by the development of EM 9 a new level of the electron microscope has been created. The type EM 9 had been improved four times during the last ten years, in a way that all the new improvements could be built into the older instruments subsequently as well. This principle permits the user to adapt his instrument to the most up-to-date level of technical advance thereby attaining a high stability of his instrument's value. A few examples of the application of this 60 kV medium class electron microscope will prove its performing capabilities (Figs 3, 4, 5 and 6).

The high capacity microscope EM 10

The guaranteed resolution of the modern high capacity instruments amounting to 3—5 Å is so close to the theoretical limit that further improvement in this respect without the lens correction of the aperature- and colour errors can hardly be imagined. A worthwhile development of a high capacity instrument would have to stress mainly technical improvements of the oper-

Fig. 6. Polyethylene, crystallized from a 0.01% Xylol-solution. These crystals are extraordinarily sensitive and are destroyed by electron irradiation. The involved deflection diagram and the interferences in the crystal show that no radiation damage has ocurred yet. 19,000 ×

ational safety, the extension of the possibilities of application and the further simplification of handling the instrument. The same guiding principles formed the basis for the development of the medium class instrument EM 9 and have been followed consistently in the creation of the new high capacity microscope EM 10. The requirements set before the development of EM 10 were as follows:

The installation demands of a high capacity instrument are very low: if no closed cooling water circulation is employed, then cooling water and a one-phase, 220 V, 25 A supply is necessary. The required area for the whole apparatus is 3 m^2.

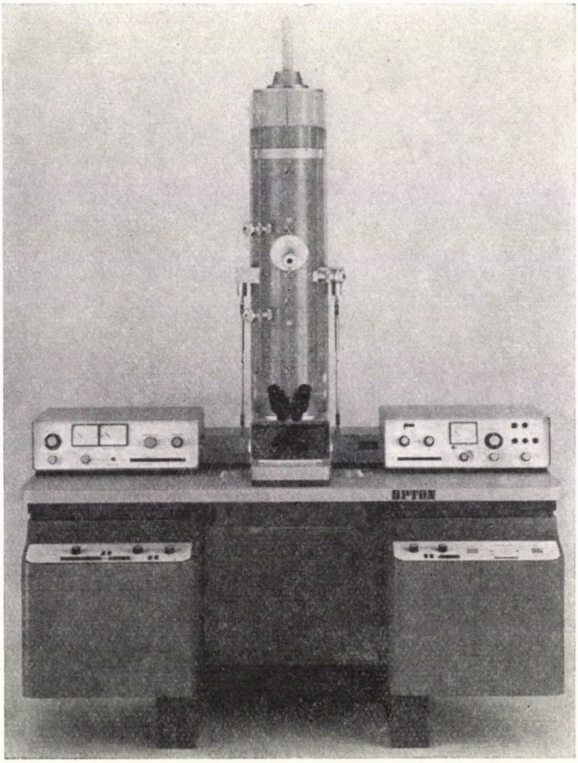

Fig. 7. High capacity electron microscope type EM 10

The vibration sensitivity of the instrument is greatly reduced by the swinging metal bearing of the column.

The guaranteed point resolution is 3,5 Å. The acceleration voltage can be adjusted between 40—100 kV in 4 stages.

The magnification is independent of the acceleration voltage and its range extends to 100×—20,000×.

Great care has been devoted to the possibility of the object manipulations to permit the user the performance of special investigations without the need of reconstructing the object chamber.

The high efficiency of the instrument demanded an efficient auxiliary sharpness adjustment aid and an automatic photographic apparatus.

Further requirements were the safe and fast assuming of working capacity after cleaning the lenses. A self-protection of the instrument against erroneous conditions has been attained to 100 per cent by automizing the most important processes. The pole shoes of all the lenses are protected by easily removable cleaning tubes in a way that the dismounting of the pole shoes is not necessary for cleaning. This is an important point, whereby in practical operation the work of adjustment is reduced to a minimum, or even eliminated.

A modern instrument is naturally fully transistorized and for reasons of miniaturization and operational safety is supplied with through-going integrated circuits. Radiation protection meeting the specifications of the recommendations of Euratom is also a natural demand.

Fig. 7 shows the instrument developed on these lines. The clear divisioning of the con-

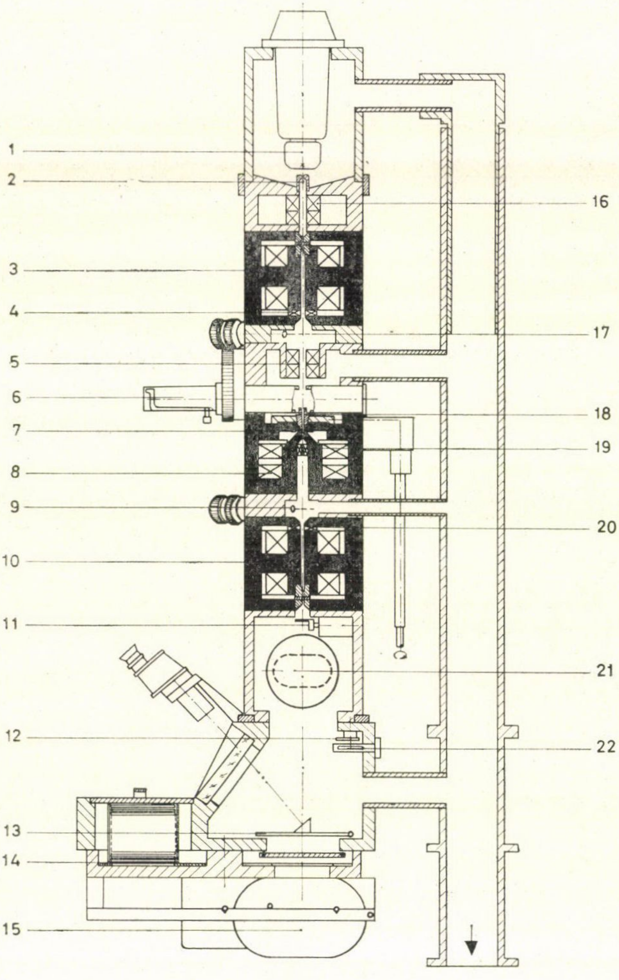

Fig. 8. Section through the column of the instrument EM 10 (1. cathode, 2. anode, 3. double condenser, 4. K_2-stigmator, 5. beam sweep system, 6. object-sluice, 7. contrast lens stop, 8. objective, 9. final range value, 10. double projector, 11. exposure shutter, 12. zero-sphalerite, 13. fluorescent screen, 14. plate-camera, 15. 70 mm roll film camera, 16. beam adjustment system, 17. lighting aperture stop of diaphragm, 18. object cartridge, 19. objective-stigmator 20. P_1-stigmator, 21. 35 mm minicamera and deflection apparatus, 22. roentgenometer tube)

trols facilitates the operation of the instrument. Four control desks serve for the arrangement of all the electrical control elements. The two desks under the table plate contain all the electrical adjustment controls.

The elements required for the routine-like operation are arranged on the two desk surfaces set on the table plate. The knobs operated frequently, as the magnification selector switch, the image brightness control, the sharpness adjustment control, the pump control and the photography installation are positioned in an easily accessible way in the direction of the column.

The column, isolated against vibration, is built up rigidly on the supporting base. The mechanical control elements on the column are reduced to a minimum. They consist of three multi-diaphragm drives, arranged in shifts to avoid confusion and of the object sluice.

Fig. 8 shows a section through the column, presenting the mechanically fixed cathode knob, a hysteresis-free beam adjustment system and two identically constructed double-lens systems, fully adaptable to the objective, employed as double condensers in the irradiation system and as double projectors in the image projection system. Between the double condenser and the objective there is an object sluice and a magnetic beam sweep system arranged. With the help of this sweep system the direction of the irradiation can be swept over the observed area of the object, e.g. for dark field microscopy. The maximum tilting angle is ±2° at 100 kV.

The astigmatic correctures of the irradiation, the image projection and the deflection (see example of a deflection diagram in Fig. 6) are cared for by three electromagnetic stigmators arranged in condenser No. 2, in the objective and in projector No. 1.

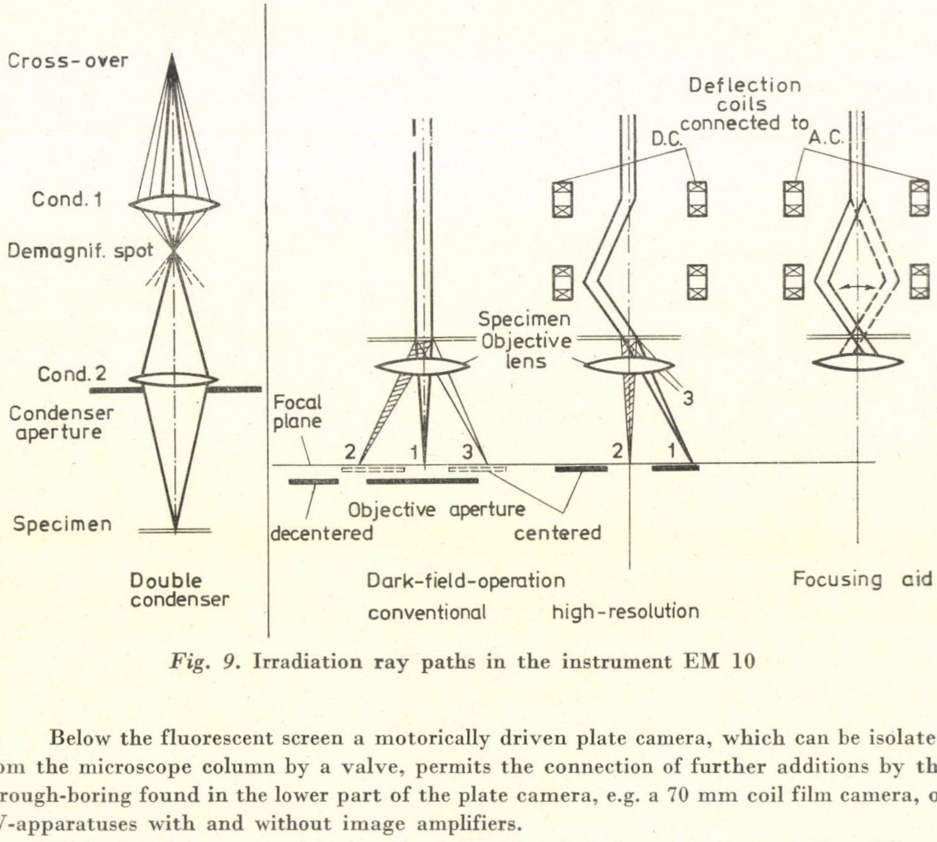

Fig. 9. Irradiation ray paths in the instrument EM 10

Below the fluorescent screen a motorically driven plate camera, which can be isolated from the microscope column by a valve, permits the connection of further additions by the through-boring found in the lower part of the plate camera, e.g. a 70 mm coil film camera, or TV-apparatuses with and without image amplifiers.

The detailed operation of the double condenser and the beam sweep system is as follows. Fig. 9 shows in its left detail drawing the principle of the double condenser. The smallest beam section of the radiation source is greatly minified by the condenser No. 1. This reduced image can be projected by the condenser No. 2 in the object plane. In this way very limited parts of the object, up to about diam. 0.7 μm, can be irradiated for attaining a high resolution.

The two central parts in Fig. 9 show two processes for dark field formation. The first process is operated with a disadjusted aperture diaphragm. In the rear focal plane of the objective the diaphragm lets the unscattered electrons pass through its bore — with bright field illumination —, whereas the scattered electrons may be captured under a greater angular incidence. If the aperture of the diaphragm is shifted for covering the primary beam, then a dark field picture is obtained, produced by the electrons scattered over the object and entering through the bore of the diaphragm. A disadvantage of this method is that the ray path is not paraxial and thereby faults are produced in the picture.

This disadvantage is avoided by the second method of dark field projection by tilting the irradiation equipment. This beam sweep system permits the adjustment of the direction of incidence in a way that the primary beam is captured by the centred diaphragm. The electrons scattered in the axial direction of the objective supply a highly resolved dark field picture produced by the paraxial beam.

A further use of the beam sweep system is as an efficient means of sharpness adjustment. For this purpose (see the drawing on the right of Fig. 9) the deflection systems are supplied by a low frequency square wave voltage, with the jumplike change of the direction of irradiation. Both directions are involved here, from which the final picture is irradiated. If the focussing plane of the objective does not coincide with the object plane, then the picture is shifted with the frequency of the directional change of the irradiation. For the better observation of these picture jumps we have selected the frequency of 3 cs. The sharpness adjustment is carried out by changing the objective current until the picture jumps cease. In the subsequent shooting this sharpness adjustment aid is naturally stopped automatically so as not to reduce the quality of the picture.

The technical requirements of the object sluice are in the case of a high capacity instrument in principle others than for a medium class instrument. Consideration must be given during the construction of the sluice to the possibilities of the multiple object manipulations, including the heating and cooling of the object.

In spite of these additional requirements we have managed to realize a simply operable, foolproof object sluice. The preparation is fixed by a threaded cap on the object cartridge, which is held in a carriage. During sluicing-in the sluice chamber is closed by rotating the eccentric part by 180°; then the sluice chamber is forepumped by moving the sluice knob along a guide link and the object cartridge is brought to the object table. The complete sluicing-in process lasts only 3 seconds.

The data of the high capacity objective are: Focal distance 2.6 mm, aperture fault constant 2.2 mm, colour fault constant 1.7 mm.

By supplying the objective with one, or two object manipulation drives, in addition to the normal object cartridge, special object cartridges can also be applied, for the purposes of extension, single sweep, double sweep and rotary sweep. The application of the multiobject cartridges facilitates the comparing investigations.

Naturally the objective is supplied with an efficient object space cooling device, whereby no object contaminations can be observed even during 15 minute irradiations by a fine beam of 1 μm diameter.

Special care has been devoted to the development of a simple magnification changeover and the automation of the photo-equipment in order to ensure the fast and rational operation with this new high capacity microscope.

The change-over of the magnification between $100\times$—$200{,}000\times$ is effected by a magnification selector switch in 25 steps. A digital magnification indication instructs the user continuously about the adjusted magnification during the observation of the picture.

The photo-apparatus is constructed on the experiences obtained during the long years of developing the instruments of type EM 9. Yet the new system of the EM 10 instruments is more universal, supplies more information and its handling is still simpler. In principle the instrument EM 10 is designed for the use of three various camera types. A camera selector switch permits the selection of the plate-, or plane film camera, the 70 mm roll film camera, or the minicamera. Each camera has its special blackening adjuster for the electron-optical blackening. In addition both big-picture cameras have a light-optical shooting data blackening

Fig. 10a. Overfocussed perforation foil, 3-times irradiated (for 1 sec each time) after 30 sec intervals. 200,000 ×

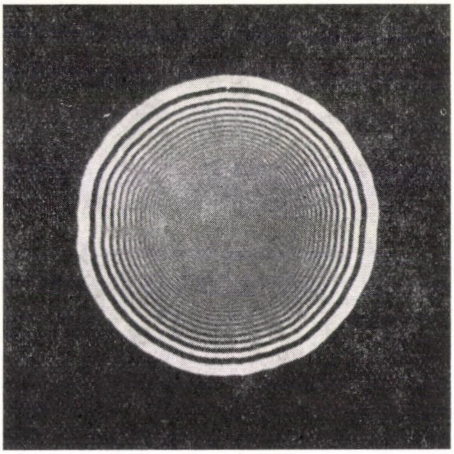

Fig. 10b. Underfocussed perforation foil; irradiated for 11 minutes with an irradiation aperture of 7.10^{-6}. 60,000 ×

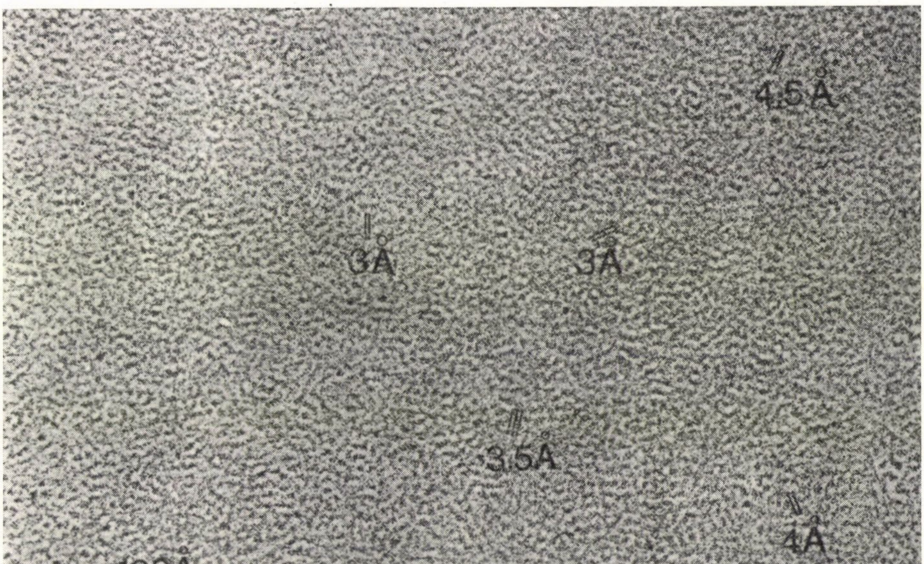

Fig. 11. Resolution test. 1.700,000 ×

adapter as well. These blackening controls must be naturally adjusted once for the plate- and film types used with the cameras. An indicator shows all the time, when a picture is on the fluorescent screen, the expectable duration of the irradiation. The time range of the irradiation automatics is between 0.2—100 seconds.

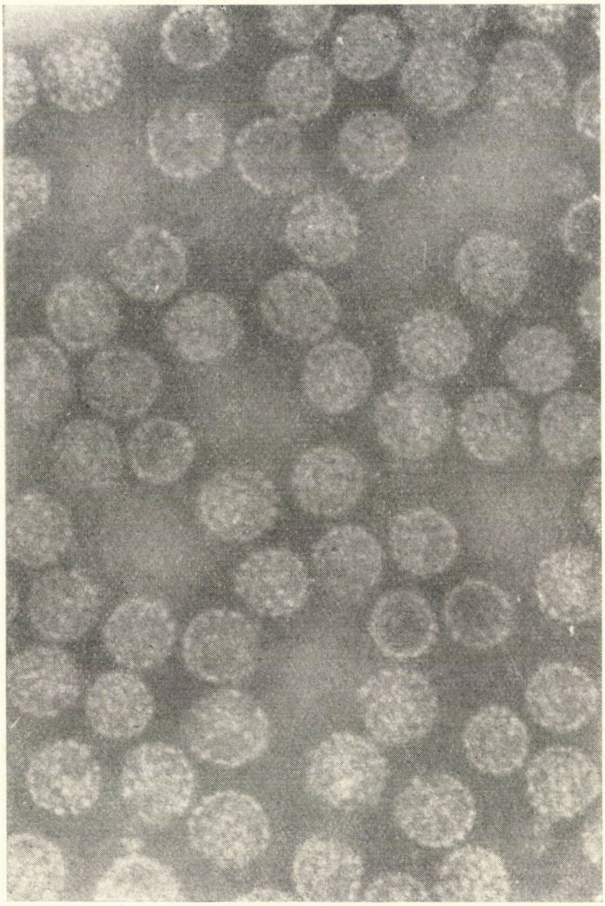

Fig. 12. Virus particle SV 40, negative painted. Prep. by: Dr. H. Frank, Max Planck Inst. for Virus Research, Tübingen. 200,000×

If the adjusted final picture brightness is too high, the irradiation meter can be controlled over. This prohibited condition for the shooting is indicated by a warnsignal lamp.

The lighting up of the green lamp contained in the shot-starting key is a free-signal, indicating that in the selected camera plates, or films are still available. Pressing down the start button starts the completely automatic photographing process. The fluorescent screen is run up and the irradiation automatics control the proper irradiation period through the lock-control. Simultaneously 5 characteristic data of the shot are printed on the edge of the negative (a five-digit serial number, the year, a freely selected recognition letter, the magnification grade and the acceleration voltage) in light-optical execution. After the irradiation period is up, the fluorescent screen closes and the shooting material is transferred further. The complete shooting cycle only takes 4 seconds, without the irradiation period.

The shooting capacity of the series plate camera amounts to 30 plates, or plane films respectively. This capacity can be considerably extended by the insertion of a 70 mm roll film camera. The maximum applicable filmlength is 5 m providing for 75 shots. A microswitch-film-feeler scans the filmstage at the entrance to indicate if still another shot is possible. This scanning and indicating process permits the inserting of arbitrarily shorter length films into the camera and their utilization without any measuring or calculation problems.

All these elaborated fast photo-installations would miss their destination if no sufficient shooting material were available in dried eondition for the replacement. The big (20 lit. capacity) dry chamber permits by its purposeful local arrangement the transfer of the photo-material into the opened storage chamber of the plate camera in the fastest possible way. Figs 10—11 show the high mechanical and electrical stability of the instrument as well as a test resolution, in which a point resolution of 3 Å is clearly recognizable.

*

Thanks on the part of the exhibiting firm are due to the Hungarian Academy of Sciences for the permission to hold this Symposium, at which the ultramicrotome -program of Messrs. C. Reichert, Vienna was also presented — in its rooms as well as to Mr. Zoltan Mátyássy, Department Head (Akadimport) for creating the organiziatonal conditions and to Mr. Emil Roósz, the interpreter of the symposium for the simultaneous interpretation.

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FUNCTIONAL ORGANIZATION OF COTYLEDON PLASTS IN THE COURSE OF EMBRYOGENESIS IN PISUM SATIVUM L

Besides studies on the ultrastructure of chloroplasts investigations into the fine structure of the leucoplasts formed in the tissues of storing organs, cotyledons and endospermium have recently come into prominence (Badenhuizen 1964, Sárkány 1966, Badenhuizen—Salema 1967, Gracza—Sárkány 1967, Gracza—Fridvalszky—Dános 1969, Gracza—Frid-

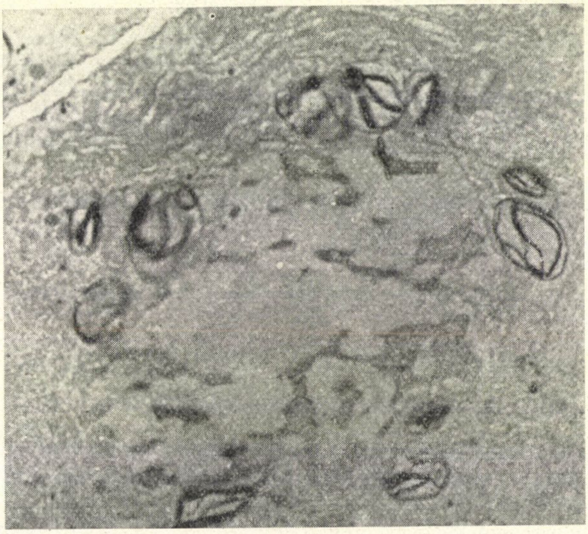

Fig. 1. Mesophyllic cell of young cotyledon with proplasts in *Pisum sativum* L

Fig. 2. Chloroplast with starch grain

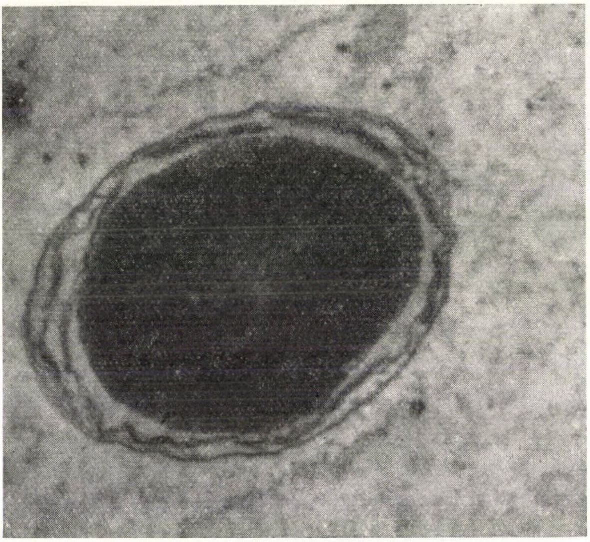

Fig. 3. Thyllacoids pressed by starch to the membrane in the chloroamyloplast

VALSZKY—SÁRKÁNY 1971, BADENHUIZEN 1971). In the present paper the fine structural conditions of the leucoplasts developing in pea cotyledons are discussed in relation with the nutrient accumulation.

Material for the light- and electron microscope studies of nutrient accumulation was collected at five stages of embryo development. At these stages the lengths of the developing cotyledons were 1.5, 3, 5, 7 and 9 mm respectively. When preparing the light microscope section series we applied Bouin-fixative and paraffin inbedding, while in the case of electron microscope studies potassium permanganate fixation and ultra-thin sections inbedded in durcupan.

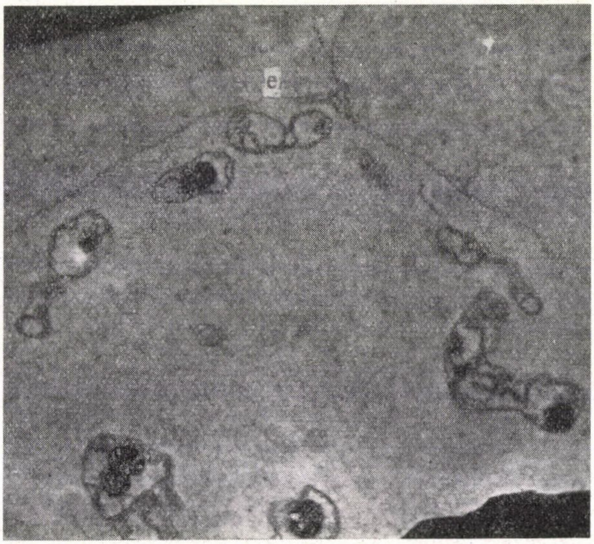

Fig. 4. Subprotodermal cell with chloroelaioplast *(e)*

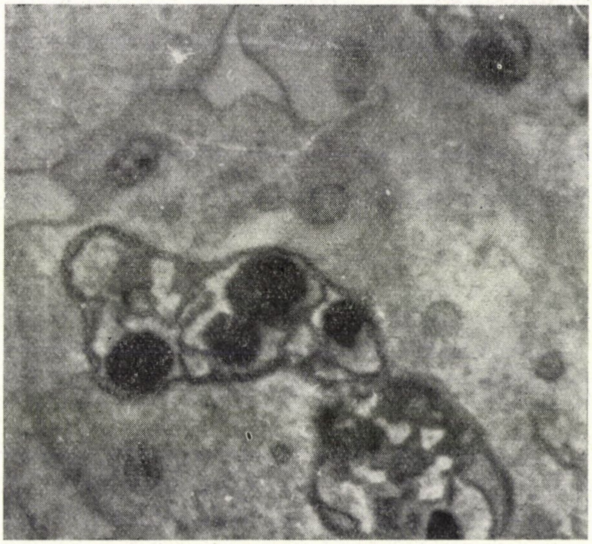

Fig. 5. Chloroelaioplast with oil bodies

The cross-section of the cotyledon primordia at the first stage of the pea embryo shows that the highly meristemic cells are square or brick shaped and tightly closed. In the cells the cytoplasmic reticulum and numerous mitochondria while only a low number of dictiosomata are appearing and in the proplasts around the nucleus the growing of the inner membrane and the development of the thyllacoids have started. At the second stage differentiation in the young chloroplasts has advanced, and stroma- and grana thyllacoids have already developed in them. Somewhat later structural and functional differentiation begins in the young chloroplasts, namely, in the inner cells of the mezophyllum of the cotyledon primordium the chloroplasts remain spherical or egg-shaped, the stroma and rich grana thyllacoids take up a position near the plast membrane (Fig. 1), and in the centre a dark body — starch — begins to appear (Fig. 2). On the other hand, in the mezophyllic cells near the protoderma the young chloroplasts become increasingly elongated and occasionally even bend, inside them small spaces develop between the stroma and grana thyllacoids which will be the places of future oil bodies (Fig. 3e).

At the next stage small spherical proplasts appear in the outer and inner cells of the mezophyllum. After a short time the development of thyllacoids also begins in them, the stroma lamellae being arranged in 1—3 concentric rings (Fig. 5p). At that time in plasts containing starch a light zone appears in the middle of the starch and becomes oval. In the subprotodermal regions, spherical electrodense oil bodies exudate in the spaces bordered by thyllacoids between the elongated chloroplasts.

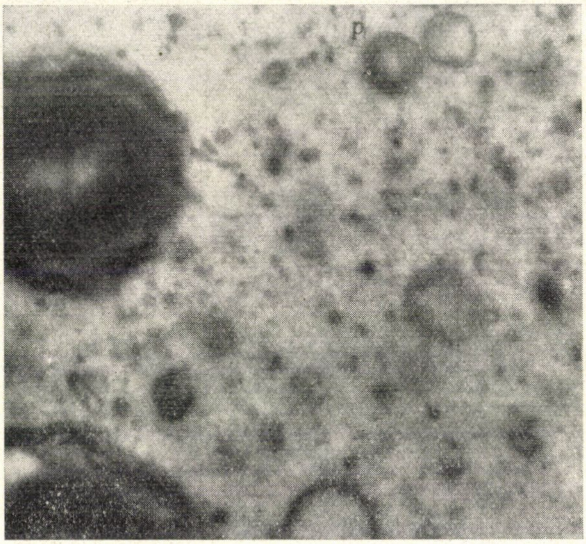

Fig. 6. Protoplast with stroma thyllacoids (*p*)

At the fourth stage the growing starch grains gradually press the thyllacoids of the chloroplasts to the membrane (Fig. 3). In the starch grains the previously starting corrosion continues in a longitudinal crack (Fig. 5), and owing to the thyllacoids left behind around the starch this structure can be considered to be a chloroamyloplast. In the other plast type the thyllacoid structure can still be observed around the oil bodies (Fig. 4 and 6.), so this is a functionally modified new organel which we suggest calling chloroelaioplast. In the proteoplasts developing later a protein-like matter appears.

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COMPARATIVE STUDY OF SOME DISINFECTANTS FROM THE POINT OF VIEW OF THE DAIRY INDUSTRY, WITH SPECIAL REGARD TO THE IODOPHORS

1. On iodophors in general. Iodine is an antiseptic of excellent action. Nevertheless, its wider use has so far been prevented by its being practically insoluble in water, having an unpleasant odour, discolouring the technical equipment and damaging skin and metallic surfaces. Shelanski discovered (cit. Twomey 1968), however, that through an interaction between polyvinyl-pyrrolidone and iodine all disadvantageous properties of the lather are reduced without its effect on the microbes decreasing. These new substances were given the name "mild iodine compounds" or "iodophors" (after the Greek word "phoros" = bearer). The "active iodine" released from the iodophors is only 30 per cent of the molecular weight in concentrated solutions, while increasing to 70—80 per cent due to dilution. As to their structure, Garrett, Schmidt-Winicow, Mayhew-Hyatt, Lawrence, McBain (cit. Twomey 1968) and Erdey-Gruz—Schay (1954) pointed out that the iodophors are micellar complexes, or in other words associated colloids. Of the surfactants used for the production of iodophors non-ionic, anionic and cationic detergents can be employed. The use of the latter is not practical, as the rising pH-value and the hardness of water have an unfavourable influence on their action. Of the non-ionic surface active substances the alkyl-phenolosy-polyglycole ethers were found to be the most suitable for iodophor production (Schmidt—Winicow, Bartlett—Schmidt, Hugo—Newton, Allawalla—Riegelman, Brost—Krupin, Siggia, Allawalla—Riegelman, Terry—Shelanski, Lazarus, Johnson, Osol—Pines, Kronick, Schick (cit. Twomey 1968). In an acidic medium iodine released from them is not retransformed into iodids as it is in an alkaline medium and thus they cannot be mixed with alkaline detergents and soaps. When dissolved in water hotter than 35 °C the iodine evaporates from the solution.

2. Effect of iodophors on metals. According to Hugo and Newton (cit. Twomey 1968) the iodophors practically only corrode copper and silver and their alloys.

3. Toxicity of iodophors. The lethal dose determined in experiments performed with guinea pigs and white rats is 4000 ppm iodine/kg. The natural iodine content of milk and meat is about 0.05 ppm. 0.1 ppm iodine carried by iodophors in the milk can be demonstrated with alpha-naphtoflavone (Kovács—Mészáros 1970, Bányai 1961), 10 ppm even by the sense organs, while 40 ppm iodine inhibits the bacterial acidification (thus it is not worth while putting it into milk with a fraudulent intention to prevent acidification or deterioration).

4. The germicide effect of iodophors. Within a pH range of 2.4—3 iodophors even when cold are effective against viruses, bacteria, saccharomyces and protozoa alike; e.g. against the virus of foot- and mouth disease at a concentration of 5 per cent (500 ppm) (SZENT-IVÁNYI 1971). The veterinarian studies connected with this were performed in Hungary by NYIREDY (1967) who found that at concentrations of 0.15 (25 ppm), 0.45 (75 ppm), 0.9 (150 ppm) and 2 per cent (300 ppm) iodine is effective against the following microbes: *E. coli*, *S. typhi murium*, *Str. dysgalactiae*, *Bact. rhusiopathiae*, *Past. multiseptica*, *Bac. subtilis*, *S. suipestifer*, *Str. agalactiae*, *Staph. aureus*, *List. monocytogenes*, *Bac. anthracis*, *Aspergillus flavus*, *Penicillium glaucum*.

5. Comparative studies of disinfectants in relation to the dairy industry. In the course of the cleaning and disinfecting operations the suitability of tools and chemicals used is an additional requirement (besides those of public health and animal hygiene) in the dairy industry.

The law-decree No. 27. 1958. para. 5. and 6. and para. 9. and 10. of decree No. 50/1958. (IX. 6.) Gov. issued for the execution of the former prescribe the observation of the above requirements.

Experts directing supervision, investigation and production must know how the conditions under which the prescribed microbiological quality and limit value can be maintained, are to be ensured, and what the means it can be attained by are, and how the tests on which the expert opinion is based are to be performed before the introduction of new disinfectants (already permitted from a sanitary point of view).

In the dairy industry the aim of cleaning and disinfection is to ensure practically sterile surfaces and conditions.

The principle of practical sterility was introduced by DEMETER (1967) to evaluate the resuóts of the cleaning and disinfecting of cans as regards lactic acid, and *E. coli* and coliform groups. The Station has extended this principle to include all microorganisms and the dairy equipment, too (CZEIDER—WAGNER 1968).

6. The concept of practical sterility. a) Pipe lines, containers and other equipment surfaces in contact with milk or milk products are practically sterile when a ml rinsing water sample taken from them after cleaning and disinfection contains only as many microorganisms as present in the water provided by the public utility works and used for rinsing, or in well-water of drinking quality.

b) Mechanically washed cans and bottles are practically sterile when less than one microorganism per 1 ml of the milk or milk product poured in is present on their total inner surface.

On practically sterile surfaces no pathogens are allowed to be present, while conditional pathogen E. coli as well as the conditional pathogens found in water (Pseudomonas) only in traces.

In the course of our investigations the following disinfectants were compared: Tego 51 (Theodor Goldschmidt AG Chemische Fabriken, Essen), tagonint (Byk-Gulden Lomberg Chem. Fabrik GmBH Konstanz-Bodensee), sodium hypochlorite (hypo, Borsod Chemical Works), nitrogenol (Enterprise of Cosmetics and Household Chemicals), Dichinol RA (Diversey), iodophors (CIBA-Phylaxia).

In Hungary three iodophor preparations are put in circulation by the firms CIBA-Phylaxia:

1. IOSAN for the sterilization of surfaces in contact with milk and milk products,
2. IOSAN CCT for the sterilization of skin,
3. WESCODYNE for the sterilization of floors, lavatories and surroundings.

During the comparison of the disinfectants the cleaning and sterilizing methods prescribed in the dairy industry were applied.

For the investigation 1 lit. milk bottles and 25 lit. aluminium and tin-plate cans were used. The total inner surfaces of cans and bottles (including the lower surface of the lids of cans touching the milk) were rinsed with fresh milk, closed and stored — cans for 24 hours, glass bottles for 72 hours — at 30 °C; then rinsed again with a known amount of sterile water, and the microorganism content of the latter was determined and referred to the total inner surface. In the course of the investigation the amounts of E. coli and coliform and of total microbes present on the surfaces were determined.

The cans and bottles were first rinsed with cold water, then washed with a 2 per cent Csepel 12 alkaline solution (similar to the composition of P_{3-12} produced by the Henkel Co.) of 65 °C temperature, and rinsed out again with water. After the removal of the Csepel 12 solution the number of microbes on the total inner surface of the cans was again determined (in the case of the glass bottles tests after the alkaline washing were dispensed with as unnecessary owing to the relatively great fineness of glass surfaces), a disinfectant of 0.1 per cent concentration was applied (instantaneous effect through rinsing) then removed by rinsing (until foaming stopped). After sterilization and 24 hours of repeated storage at 30 °C the amount of microorganisms on the total inner surface was again determined in order to check the result of sterilization and the subsequent reproduction (Pünter 1969) (Tables 1, 11 and 15).

The tests were performed according to the prescriptions of the standard MSZ 3743. Subsequently we studied the effect of disinfectants on *Pseudomonas aeruginosa* important from the point of view of the dairy industry. For the examination a 24 hours old broth culture of more than 10^6/ml microbe content, inoculated on three successive days, was used. The concentrations of the disinfectants were 1, 0.1. per cent; the time of action: instantaneous, 10 minutes, 1 hour and 2 hours, respectively. The effect of disinfectants was studied in distilled water solutions and in the presence of 10 per cent milk at room temperature; then the growth of the microbes was examined in a culture medium of indicator and milk-sugar content after 24 hours breeding at 37 °C (Table 12). Tego 51 (except for the new preparations), tagonin, and nitrogenol are perfectly ineffective against Pseudomonas aeruginosa, therefore we do not present tables of the results of the investigations.

Table 1

Sterilization of tin-plate cans with Tego 51

Marking of cans	Microbe content of the total inner surface									
	before previous rinsing		after alkaline washing		sterilization with Tego 51		subsequent reproduction		control sterile distilled water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
I.	10^7	10^9	10^4	10^6	0	10^3	10^3	10^6	0	0
II.	10^7	10^9	10^5	10^7	0	10^3	10^5	10^6	0	0
III.	10^8	10^{11}	10^5	10^8	0	10^4	10^5	10^6	0	0
IV.	10^6	10^9	10^4	10^7	0	10^5	10^3	10^7	0	0
V.	10^7	10^9	10^5	10^6	0	10^4	10^4	10^6	0	0
Sterilization of aluminium cans with Tego 51										
VI.	10^7	10^9	10^4	10^7	0	10^6	10^7	10^8	0	0
VII.	10^7	10^9	10^5	10^7	0	10^4	10^3	10^5	0	0
VIII.	10^8	10^9	10^5	10^7	0	10^4	10^3	10^5	0	0
IX.	10^8	10^9	10^5	10^7	0	10^6	10^4	10^6	0	0
X.	10^7	10^{10}	10^4	10^8	0	10^6	10^4	10^8	0	0

Table 2

Sterilization of tin-plate cans with Tagonin

Marking of cans	Microbe content of total inner surfaces									
	before previous rinsing		after alkaline washing		after sterilization with tagonin		subsequent reproduction after 24 hours		control sterile distilled water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XI	10^8	10^{11}	10^4	10^5	10^4	10^5	above 10^6	above 10^7	0	0
XII	10^8	10^{11}	10^5	10^6	10^5	10^5	above 10^6	above 10^7	0	0
XIII	10^8	10^{10}	10^5	10^6	10^5	10^6	above 10^6	above 10^7	0	0
XIV	10^8	10^{11}	10^5	10^6	10^4	10^6	above 10^6	above 10^7	0	0
XV	10^8	10^{11}	10^5	10^6	10^4	10^6	above 10^6	above 10^7	0	0
Sterilization of tin-plate cans with Tagonin										
XVI	10^8	10^{11}	10^5	10^6	10^4	10^6	above 10^6	above 10^7	0	0
XVII	10^8	10^{11}	10^4	10^6	10^5	10^6	above 10^6	above 10^7	0	0
XVIII	10^8	10^{11}	10^4	10^6	10^4	10^5	above 10^6	above 10^7	0	0
XIX	10^8	10^{10}	10^5	10^5	10^4	10^5	above 10^6	above 10^7	0	0
XX	10^8	10^{11}	10^4	10^6	10^5	10^6	above 10^6	above 10^7	0	0

Table 3

Sterilization of tin-plate cans with Nitrogenol

Marking of cans	Microbe content of total inner surfaces									
	before previous rinsing		alkaline washing		after sterilization with nitrogenol		subsequent reproduction after 24 hours		control sterile distilled water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XXI	10^8	10^{12}	10^5	10^6	0	10^2	10^6	10^7	0	0
XXII	10^8	10^{11}	10^5	10^6	0	10^3	10^6	10^7	0	0
XXIII	10^7	10^9	10^4	10^6	0	10^3	10^4	10^6	0	0
XXIV	10^5	10^9	10	10^7	0	10^3	10^3	10^5	0	0
XXV	10^7	10^{11}	10^5	10^6	0	10^2	10^6	10^7	0	0
Sterilization of aluminium cans with Nitrogenol										
XXVI	10^8	10^{10}	10^4	10^6	0	10^3	10^6	10^7	0	0
XXVII	10^8	10^{10}	10^4	10^5	0	10^3	10^6	10^7	0	0
XXVIII	10^8	10^9	10^5	10^7	0	10^4	10^4	10^5	0	0
XXIX	10^7	10^9	10^4	10^7	0	10^3	10^4	10^5	0	0
XXX	10^7	10^9	10^5	10^7	0	10^2	10^3	10^5	0	0

Table 4

Sterilization of tin-plate cans with sodium hypochlorite

Marking of cans	Microbe content on total inner surfaces									
	before previous rinsing		after alkaline washing		after sterilization with sodium hypochlorite		subsequent reproduction after 24 hours		control sterile distilled water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XXXI	10^7	10^8	10^5	10^7	0	10	0	10^3	0	0
XXXII	10^8	10^9	10^5	10^6	0	10^2	0	10^3	0	0
XXXIII	10^7	10^{10}	10^5	10^8	0	10^2	0	10^3	0	0
XXXIV	10^6	10^{10}	10^4	10^8	0	10^2	0	10^3	0	0
XXXV	10^5	10^8	10^3	10^5	0	10	0	10^3	0	0
Sterilization of aluminium cans with sodium hypochlorite										
XXXVI	10^6	10^9	10^5	10^7	0	10	0	10^2	0	0
XXXVII	10^7	10^9	10^5	10^7	0	10	0	10^2	0	0
XXXVIII	10^6	10^9	10^4	10^6	0	10^2	0	10^3	0	0
XXXIX	10^8	10^{10}	10^6	10^8	0	10^2	0	10^3	0	0
XL	10^6	10^9	10^5	10^7	0	10^2	0	10^3	0	0

Table 5

Sterilization of tin-plate cans with Iosan

Marking of cans	Microbe content on the total inner surface									
	before previous rinsing		after alkaline washing		after sterilization with Iosan		subsequent reproduction after 24 hours		control sterile distilled water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XLI	10^8	10^9	10^4	10^6	0	10^3	0	10^5	0	0
XLII	10^8	10^9	10^5	10^7	0	10^2	0	10^4	0	0
XLIII	10^{10}	10^{11}	10^5	10^8	0	10^3	0	10^6	0	0
XLIV	10^8	10^9	10^5	10^6	0	10^2	0	10^5	0	0
XLV	10^8	10^9	10^5	10^6	0	10^2	0	10^4	0	0
Sterilization of aluminium cans with Iosan										
XLVI	10^8	10^{10}	10^4	10^6	0	10^2	0	10^4	0	0
XLVII	10^9	10^{11}	10^5	10^7	0	10^3	0	10^5	0	0
XLVIII	10^4	10^{10}	10^5	10^7	0	10^3	0	10^3	0	0
XLIX	10^4	10^{10}	10^4	10^7	0	10^3	0	10^4	0	0
L	10^4	10^9	10^4	10^6	0	10^2	0	10^5	0	0

Table 6

Sterilization of 1 lit. glass bottles with Tego 51
(earlier preparation)

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
I	10^7	10^8	0	10^2	0	10^3	0	0
II	10^7	10^8	0	10^3	0	10^3	0	0
III	10^6	10^8	0	10	0	10^2	0	0
IV	10^6	10^7	0	10^3	0	10^5	0	0
V	10^5	10^8	0	10^2	0	10^4	0	0

Table 7

Sterilization of 1 lit. glass bottles with Tagonin

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
VI	10^7	10^8	0	10^4	10^5	10^6	0	0
VII	10^5	10^7	0	10	10^4	10^6	0	0
VIII	10^6	10^7	0	10^3	10^4	10^6	0	0
IX	10^6	10^8	0	10^3	10^3	10^7	0	0
X	10^7	10^9	0	10^4	10^5	10^7	0	0

Table 8

Sterilization of 1 lit. glass bottles with Nitrogenol

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XI	10^4	10^7	0	10	0	10^2	0	0
XII	10^4	10^7	0	10^2	0	10^3	0	0
XIII	10^6	10^7	0	10^2	0	10^3	0	0
XIV	10^7	10^9	0	10	0	10^2	0	0
XV	10^7	10^9	0	10	0	10^2	0	0

Table 9

Sterilization of 1 lit. glass bottles with sodium hypochlorite

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XVI	10^7	10^8	0	10	0	10^2	0	0
XVII	10^7	10^8	0	0	0	10	0	0
XVIII	10^5	10^6	0	0	0	10	0	0
XIX	10^6	10^7	0	10	0	10^2	0	0
XX	10^6	10^7	0	10	0	10^2	0	0

Table 10

Sterilization of 1 lit. glass bottles with Iosan

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XXI	10^4	10^7	0	10	0	10	0	0
XXII	10^4	10^7	0	10	0	10^2	0	0
XXIII	10^5	10^7	0	10	0	10^2	0	0
XXIV	10^4	10^8	0	below 10	0	10	0	0
XXV	10^4	10^9	0	10	0	10^2	0	0
XXVI	10^5	10^7	0	10	0	10^2	0	0
XXVII	10^5	10^7	0	10	0	10^2	0	0
XXVIII	10^4	10^7	0	10	0	10^2	0	0
XXIX	10^4	10^7	0	10	0	10^2	0	0
XXX	10^5	10^7	0	10	0	10^2	0	0

Hypomycetes and *Saccharomycetes* of mixed flora were studied under conditions similar to those described above, and growth was checked after 72 hours of breeding on lactic acid malt agar at room temperature (Tables 13—14).

As a result of our investigations we have established that in sterilizing glass surfaces iodophors and nitrogenol have the same effect, as practical sterility can be attained with either of them.

Tego 51 and tagonin are unsuitable for use in the dairy industry. The application of sodium hypochlorite and of preparations with active chlorine content (Dichinol RA) is conditioned by veterinarian instructions.

Table 11

Washing and sterilization of 1 lit. glass bottles with 1 per cent Dichinol RA

Marking of bottles	Microbe content on total surface							
	before previous rinsing		after washing with alkaline and sterilization		subsequent reproduction after 24 hours		control sterile water	
	coli	total microbe	coli	total microbe	coli	total microbe	coli	total microbe
XXXI	10^7	10^9	0	0	0	0	0	0
XXXII	10^7	10^9	0	0	0	0	0	0
XXXIII	10^7	10^9	0	0	0	0	0	0
XXXIV	10^7	10^9	0	0	0	0	0	0
XXXV	10^7	10^9	0	0	0	0	0	0
XXXVI	10^7	10^9	0	0	0	0	0	0
XXXVII	10^7	10^9	0	0	0	0	0	0
XXXVIII	10^7	10^9	0	0	0	0	0	0
XXXIX	10^7	10^9	0	0	0	0	0	0
XL	10^7	10^9	0	0	0	0	0	0

Table 12

Effects of iodophors (Iosan, Iosan CCT, Wescodyne) and sodium hypochlorite on Pseudomonas aeruginosa

Time	Control	Iodophors		Iodophors + 10% milk		Sodium hypochlorite		Sodium hipochlorite + 10% milk		Dichinol RA		Dichinol RA + 10% milk	
		1%	0.1%	1%	0.1%	1%	0.1%	1%	0.1%	1%	0.1%	1%	0.1%
Instantaneous	++++	Ø	++++	Ø	++++	Ø	+++	++	++++	Ø	Ø	Ø	++++
10 minutes	++++	Ø	++++	Ø	++++	Ø	Ø	Ø	++++	Ø	Ø	Ø	++++
1 hour	++++	Ø	+++	Ø	++++	Ø	Ø	Ø	++++	Ø	Ø	Ø	++++
2 hours	++++	Ø	++	Ø	++++	Ø	Ø	Ø	++++	Ø	Ø	Ø	++++

++++ = very strong growth
+++ = strong growth
++ = moderate growth
\+ = poor growth
Ø = no growth

Table 13

Effects of iodophors (Iosan, Iosan CCT, Wescodyne) and nitrogenol on hypomycetes (mixed plant flora)

Time	Control	1 percent solution of iodophors	0.1% solution of iodophors, and 1, 0.1% solutions with 10% milk present	1, 0.1% solutions of nitrogenol, and the same concentration with 10% milk present
Instantaneous action	++++	+++	++++	++++
10 minutes	++++	++	++++	++++
1 hour	++++	∅	++++	++++
2 hours	++++	∅	++++	++++

++++ = very strong growth
+++ = strong growth
++ = moderate growth
\+ = poor growth
∅ = no growth

Table 14

Effects of iodophors (Iosan, Iosan CCT, Wescodyne) and nitrogenol on saccharomycetes (mixed plant flora)

Time	Control	1% solution of iodophors	0.1% solution of iodophors, and 1, 0.1% solutions with 10% milk present	1% solution of nitrogenol	0.1% solution of nitrogenol	1, 0.1% solutions of nitrogenol with 10% milk present
Instantaneous action	++++	∅	++++	∅	±	++++
10 minutes	++++	∅	++++	∅	∅	++++
1 hour	++++	∅	++++	∅	∅	+++
2 hours	++++	∅	++++	∅	∅	++

++++ = very strong growth
+++ = strong growth
++ = moderate growth
\+ = poor growth
± = very poor growth
∅ = no growth

On tin-plate and aluminium surfaced cans the iodophors are more effective; additional reproduction after 24 hours is of lower extent, and as a contrast to the application of nitrogenol *E. coli* and coliform practically cannot be found on the surfaces even after 24 hours, which is important for the guaranteed time of the milk.

The 1 per cent solution of iodophor immediately destroys *Pseudomonas aeruginosa* both in a medium of distilled water and in the presence of 10 per cent milk even in the case of an instantaneous action, while nitrogenol is totally ineffective against it.

In a protein-free medium 1 per cent iodophor solutions destroy the saccharomycetes immediately, and the hypomycetes within an hour.

Table 15

Bacterium number in post-rinsings after washing with 2 per cent Csepel 12 and 0.1 per cent Iosan solutions of 65 °C temperature and sterilization

Post-rinsing samples from a dairy equipment	E. coli and coliform	Total microbe	Evaluation
Rinsings from a 2500 lit. pre-storing container	0	10^2	practically sterile
Rinsings from a 5000 lit. pre-storing container	0	10^2	practically sterile
Rinsings from a 10.000 lit. post-storage container No. I.	0	10^2	practically sterile
Rinsings from a 10.000 lit. post-storage container No.II.	0	10^2	practically sterile
Rinsings from a 10.000 lit. post-storage container No.III.	0	10^2	practically sterile
Rinsings from a 10.000 lit. post-storage container No.IV.	0	10^2	practically sterile
Rinsings from a 10.000 lit. post-storage container No.V.	0	10^2	practically sterile
Rinsings from a milk pipe-line	0	10^2	practically sterile
Rinsings from the filling drum No. I. of a Graham Enock machine	0	10^2	practically sterile
Rinsings from the filling drum No. II. of a Graham Enock machine	0	10^2	practically sterile
Rinsings from tank No.I. of a tank lorry	0	10^2	practically sterile
Rinsings from tank No. II. of a tank lorry	0	10^2	practically sterile
Rinsings from tank No. III of a tank lorry	0	10^2	practically sterile
Tap water	0	10^2	practically sterie

Nitrogenol has no sufficient effect in practice on the hypomycetes, while its 0.1 per cent solution is effective against saccharomycetes within 10 minutes.

When comparing the bactericide effect of iodophors with that of preparations with active chlorine content we find that the iodophors show the same efficiency at a lower active iodine concentration. E.g. in attaining practical sterility under the conditions of dairy plants, the effect of 16.6 ppm active iodine is identical with that of 99 ppm active chlorine.

An additional advantage of the iodophors is that they are indicators for themselves, namely: when the brown colour of their aqueous solutions turns into amber their effect decreases, when they become colourless they are completely ineffective — as observed by us, too. The exhaustion of nitrogenol cannot be demonstrated in such a simple way under operative conditions.

Prepared at the Dairy Trust, Control Station of Dairy Products, Budapest.

A. Wagner

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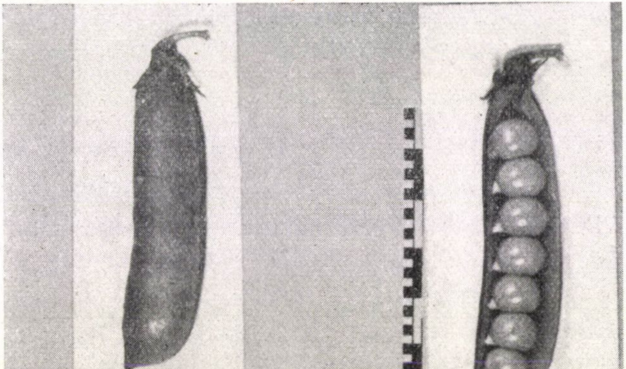
PEA VARIETY UJMAJORI KORAI VIKTORIA



Taxonomical place: Pisum sativum L. convar. *vulgare* ALEF. var. *superfluens* ALEF.

Origin: from a commercial product by individual selection

Beginning of breeding: 1947, Ujmajor

State qualification: state certified improved variety, 1959; first accepted 1955

Breeder: Dr. A. Ács, Debrecen

General characterization: earliest of the Victoria peas, a husking pea of reliable yield (KAPÁS *et al.* 1965)

Morphological description:

Root system: penetrating medium deep into the soil

Shoot system: vigorous; initial development rapid

Stem: 80—120 cm high, light green, angularly costate; internodes are thick, 12—14 in number; often procumbent

Foliage: the large leaves are loosely set on the stalk, their colour is yellowish green; the leaflets are broad, egg-shaped, smooth-edged with the apex rounded off and ending in a beard-like projection (this may be absent); tendril medium large.

Flowers: mostly singly but sometimes in pairs on the floral axis; corolla white, standard smooth-edged (at the most slightly ondulate), the base of the standard V-shaped, its tip ending in a sharp tooth. Wing blades are round.

Legume: light green, broad, straight with a blunt tip; its length is 5—8 cm, width 1.2—1.8 cm; 4—6 seeds develop in it. 6—14 legumes grow on a plant.

Seed: light green when unripe and yellow with a pink shade when ripe; large, round, smooth surfaced. Thousand-grain-weight 250—340 g.

Biological character:

Vegetation period: from emerging to flowering 37—51 days, from flowering to ripening 34—44 days; total vegetation period 73—96 days (IVÁNYI 1966); early ripening (earliest of the similar varieties); ripening can be expected between 4th and 23rd July.

Water requirement: low; however soils of good water regime are preferable.

Resistance to disease: disease resistance satisfactory, and due to its early ripening pests cannot attack it either.

Farm technology requirement:

Seeding: best in March (at the very beginning of spring)

Soil requirement: sufficiently supplied with nutrients; the plant soon covers the soil and suppresses the weeds (KAPÁS *et al.* 1965).

Productivity: the several years average yield: 10.8 q/cad.yoke (1 cad.yoke = 1, 422 acres); crude protein content as a percentage of the dry matter: 26.8 percent (IVÁNYI 1966).

Region of cultivation: In Hungary it can be successfully grown in Transdanubia, in the northern parts of the country and in the Great Plain.

Prepared at the Department of Botany, University of Agrarian Sciences, Debrecen.

Gy. MÁNDY

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FORUM

ON THE CHEMICAL NATURE OF "PROMINE" AND "RETINE"

By

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The authors found freely methylated basic amino acid derivates in calf thymus and calf liver originally studied by Szent-Györgyi et al., of which they identified ε-N-trimethyl-lysine and N^G, N^G-dimethyl-arginine. On a methodological basis and on the grounds of partial biological investigation results already published the authors proved the identity of methylated basic protein-amino acids with "promine" and "retine."

Introduction

The growth promotion or — inhibition of malignant tumours by certain unidentified components of normal cells is a fact that has been known for a long time (Schroeder—Cook 1939, Kelly—McDowell 1948). Studies in this subject were considerably extended by Szent-Györgyi who, taking his earlier conclusions and partial experimental results (Szent-Györgyi 1960) in consideration—gave account, first in a lecture (Szent-Györgyi—Hegyeli 1962) then in a detailed publication (Szent-Györgyi *et al.* 1962), of two biologically active substances found in calf thymus, one of which promoted the growth of malignant tumours of animal origin and was named promine, while the other, called retine, retarded it, and "the result depended on their balance." They did not succeed in isolating — and consequently in identifying — the two antagonistic substances, nevertheless, on the basis of papers published on this question (Szent-Györgyi—Hegyeli 1962, Szent-Györgyi *et al.* 1962, Hegyeli *et al.* 1963) some remarkable characteristics of the substances can be given:

a) In a highly acidic medium (2—3n HCl) the substances can be precipitated with Reinecke salt. b) The substances are of low molecular weight, and their physical and chemical features are very similar; nevertheless they can be separated by paper chromatography and characterized. In the case of identification by paper chromatography the presence of choline and betaine was a hindrance. c) Retine has the special characteristic of containing one or more unstable groups and showing a higher sensitivity to alkalis than to acids.

This preliminary characterization of the substances is the main result of the first phase of the research on promine-retine. The second phase started with the publication of Együd (1965) who on the basis of the infra-red spectra of preparations isolated by Hegyeli and himself raised the possibility that retine was a methyl-glyoxal (I) derivative of nitrogen content which was able to form reineckate. Subsequently Együd—Szent-Györgyi (1965) studied the effect of methyl-glyoxal and some of its derivatives on bacteria and generally found an inhibition of cell division. At the same time attempts were again made to isolate retine. First Együd *et al.* (1967) isolated a compound from calf liver in the form of osazone which they called retine. Fodor et al. pointed out that this compound was identical with 3-deoxy-D-glucosulose (II). According to Szent-Györgyi (1967) this compound proved inactive on tumours. As seen from these investigations the isolation of promine was pushed into the background. At that time

Szent-Györgyi *et al.* (1967) regarded retine as some glyoxal derivative, a substrate of the glyoxalase enzyme, that is, promine was considered to be this enzyme or its izomers.

The third phase of the promine-retine research consisted of those investigations in which the authors starting from the failure in isolating promine and retine wished to clarify in the first place the chemical nature of retine. **Marmasse** (1966)— when studying the chemical nature of retine — arrived at the conclusion that it was a diketene of N-content (III). According to **Fodor** *et al.* (1967) the stability of this compound in aqueous solutions is improbable. **Edgar** (1969) made a remarkable suggestion namely, that dehydro-ascorbic acid (IV) may be as important an inhibitor as imagined by Szent-Györgyi, the more so because tumour inhibition by this compound had already been described by others (**Heise** *et al.* 1957). A large number of glyoxalase inhibitors have recently been synthetized by **Vince—Wadd** (1969) and **Vince—Daluge** (1971) in order to find some chemotherapeutically important ones among them (**French—Freedlander** 1958).

The "methylating complex" of red beet *(Beta vulgaris* var. *conditiva)* was reported as having an influence on tumours in a lecture and publication by **Tyihák** (1964a,b). Tumour retardation by extracts prepared from certain organs of plants belonging to this taxon had already been reported in earlier publications (**Ferenczi** 1955, **Taylor** *et al.* 1956). During our own investigations in quaterner amin fractions prepared from red beet several unknown Dragendorff- and ninhydrin positive substances were demonstrated. Detailed examinations proved that they were ε-N-methylated lysines and guanidino-methylated arginines (**Tyihák** 1972). Parallel with the former examinations basic proteins either retarding or promoting the tumour growth could be isolated from various parts of the red beet, in which ε-N-methylated lysines and methylarginines of some tenth mole percent could also be found (**Tyihák—Szende** 1967—1970, **Tyihák** 1972).

In the present paper we report on having found free methylated basic amino acid derivatives in calf thymus originally studied by **Szent-Györgyi—Hegyeli** (1962) and **Szent-Györgyi** *et al.* (1962) and calf liver too, further, on a methodological basis and on the grounds of partial biological investigation results already published try to prove that methylated basic protein-amino acids are identical with promine and retine.

Material and Method

Thymus gland and liver from six weeks old calves were made available by the Meat Industry Enterprise of Budapest. The fresh products were used after cleaning and freezing.

Ion exchangers. Amberlite IR 120 (100—200 mesh); Dowex 50×8 (20—50 mesh). Layers. Spread layers of MN-cellulose powder 300 and Silica Gel G, and precoated plates: MN-Polygram Cel 300 and Fixion 50×8 (Chinoin, Budapest). Authentic materials. DL-N^{ε}-monomethyl-lysine HCl (MML), DL-N^{ε}-dimethyl-lysine HCl (DML) and DL-N^{ε}-trimethyl-lysine 2 HCl (TML) produced earlier by total synthesis (**Puskás—Tyihák** 1969, **Puskás—Tyihák** 1970), N^{G}-monomethyl-arginine. di-p-hydroxyazobenzol-p'-sulphonate (MMA), N^{G}, N^{G}-dimethyl-arginine. di-p-hydroxy-azobenzol-p'-sulphonate (DMA), N^{G}, N'^{G}-dimethylarginine, di-p-hydroxyazobenzol-p'-sulphonate (DMA'), 1-methyl-histidine, 3-methyl-histidine, choline and betaine.

Production of reineckates of amino acids and amines. 10—10 mg amino acid or amine was dissolved in 10—10 ml 6 n HCl, then 10—10 ml 2 per cent aqueous solution of Reinecke-salt was added to it at a ratio of 1 : 1. The mixed solution was halved; one half was kept at room temperature, the other in a refrigerator both for 12 hours.

Enrichment of aliphatic basic amino acids and their derivatives from calf thymus and calf liver. 500 g of both thymus and liver of six weeks old calves were pulpified first with 600 ml cold (0 °C) 0.9 per cent salt solution for 20 minutes. The cold pulps (0—4 °C) were filtered through a close-woven linen, then the solutions obtained centrifuged with 8000 r.p.m. at 0 °C temperature. This procedure was repeated with 400 ml each of 0.9 per cent salt solution. Cold 12 n hydrochloric acid was added at a ratio of 1 : 1 to the translucent solutions of the joint supernatant of 1000 ml each, the precipitate was centrifuged for 5 minutes at 4000 r.p.m., and a 2 per cent aqueous solution of Reinecke-salt added to the supernatant at a ratio of 1 :1. After 30 minutes the precipitate obtained at room temperature was rapidly filtered. The filtrate was placed in a refrigerator, where — after one night of standing — a precipitate again appeared and was filtered. The filtered precipitates were each washed with 10 ml cold 3 n hydrochloric acid then 50 ml n-hexane. In this way two precipitates were obtained from both the thymus and the liver of the calf, which were labelled BT-I, BT-II and BM-I, BM-II, respectively. The precipitates were each dissolved in 50 ml acetone, and the separated insoluble protein-like substances filtered through a G-3 filter. 50 ml distilled water was added to each of the filtrates, then each sample was shaken with 3×50 ml peroxide-free ether. The aqueous solutions thus freed from Reinecke-salt were carefully evaporated, and the residues used for the subsequent analytical studies.

Amino acid-analysator methods. IC—R—L resin was used for the analyses performed in a Jeol make JLC—5AH type automatic analysator. The minimum material requirement of the instrument is less than 10^{-9} mole. The single-column method was used for a complete analysis. In this case the temperature of the column at the beginning of the analysis was 40 °C for some 25', then 60 °C. When only the basic amino acids were examined, 0.35 n citrate buffer was used on a 10 cm resin column, at a column temperature of 25 °C and pH-value of 6.5. Buffer velocity was about 100 ml/hour in every case.

Paper chromatographic method. We reproduced the method elaborated by SZENT-GYÖRGYI *et al.* (1962). The following reagents were used for identification: a) ninhydrin reagent: 0.5 g ninhydrin and 0.05 g copper sulphate in 100 ml acetone. b) Dragendorff reagent: the so called Dragendorff reagent containing ethyl acetate was used in a composition suggested by VÁGUJFALVI (1960), and sensitivization erformed with 0.1 n sulphuric acid solution was also efficient (VÁGUJFALVI 1960).

Partition and adsorption layer chromatographic methods. The Silica Gel G layers were spread (Desaga apparatus) 250 μ thick then activated for one hour at 110 °C. The layers of MN-cellulose powder 300 were also spread 250 μ thick and left to dry for one night. Solvent mixtures: 1) n-butanol saturated with n-hydrochloric acid; 2) chloroform-methanol-25% NH_3 (4 : 4 : 1, V/V/V); 3) n-butanol-glacial acetic acid-water (4 : 1 : 5). Reagents: the reagents described in the paper chromatographic method, but the dilution of the Dragendorff-reagent and sensitivization with sulphuric acid were carried out in the earlier described way (TYIHÁK—VÁGUJFALVI 1970).

Ion-exchange layer chromatographic methods. Analytical methods. The Fixion 50×8 ion-exchange layer was equilibrated over 24 hours with the following buffer: 1.4 g citric acid +0.8 g NaOH + 1.2 ml HCl 37% (sp.gr. 1.19) in 1000 ml final volume (pH = 3.28). The following two citrate buffers were used as eluting mixtures: 1) 40 g citric acid. H_2O + 25 g NaOH + 5 ml HCl 37% (sp.gr. 1.19) in 500 ml final volume: 2) 50 g citric acid. H_2O + 30 g NaOH + 7 ml HCl 37% (sp.gr. 1.19) in 500 ml final volume. Eluting was carried out at room temperature or at 50 °C. An acetonous ninhydrin reagent (as earlier) was used as developing reagent (70 °C). Preparative method. Samples to be examined were applied in strips on Fixion 50×8 layers, and the 2) citrate buffer was used as eluting mixture. The edges of layers were developed with ninhydrin reagent, then on the basis of the strips appearing due to heating the layer zones to be scraped off were marked. The basic amino acids were eluated with 3 M NH_4OH solution from the scraped off dust layer, concentrated, then dissolved in methanol and dripped on different layers for identification.

Purification of basic amino acids and their derivatives on an ion exchange column

(2 × 30 cm). The samples were applied in distilled water on the H^+ form resin column. The column was washed first with distilled water, then with NH_4OH solution of increasing molarity. The basic amino acids and their derivatives were found in a relatively enriched state in fractions obtained with NH_4OH solutions of 2.0 and 3.0 mole. Small parts of samples BT and BM were applied on the H^+ form Amberlite IR-120 column (10 × 10.5 cm), and eluation was carried out first with distilled water, then with 1 M pyridine solution. The basic amino acids and their derivatives were ultimately eluated with 3 M NH_4OH.

Results

Study on the reineckate forming ability of amino acids and amines. The ability of 29 amino acids with peptide bonds too, as well as of choline and betaine to form precipitates or crystals with Reinecke-salt ($NH_4Cr(NCS)_4(NH_3)_2H_2O$) was studied in a highly acidic medium (3 n HCl). According to the study, the reineckate forming ability of amino acids is very different; this ability is especially great in the basic amino acids, and even more in their methylated derivatives. Therefore, under given conditions the latter can be highly enriched by reineckate formation from an amino acid mixture.

Paper chromatographic results. Fig. 1 presents schematically the results of our investigations made with the method used by Szent-Györgyi *et al.* (1962) in studying promine and retine. Accordingly, lysine and its three ε-N-methylated derivatives, but first of all the ε-N-trimethyl-lysine, remain practically at the starting point, like promine. On the other hand, arginine and its guanidino-methylated derivatives have a higher R_f value. Symmetric dimethylarginine has the highest R_f value falling within the R_f-value range given for retine. Fig. 1

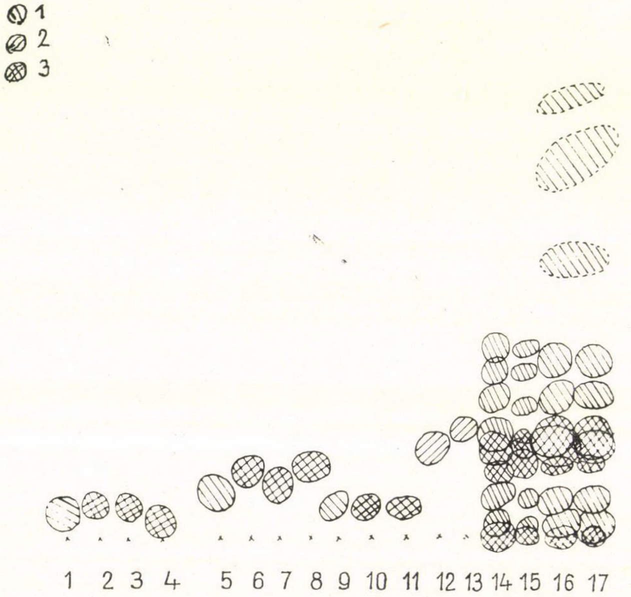


Fig. 1. Comparative study on components of calf thymus- and liver extracts separated with Reinecke-salt (Whatman 3 paper, solvent: n-butanol saturated by n HCl) 5—5 μg (1) lysine (2) MML (3) DML (4) TML (5) arginine (6) MMA (7) DMA (8) DMA' (9) histidine (10) 1-Me-His (11) 3-Me-His (12) choline (13) betaine; 0.01—0.01 ml (14) BT-I (15) BT-II (16) BM-I (17) BM-II. 1. ninhydrin-positive; 2. Dragendorff-positive; 3. ninhydrin- and Dragendorff-positive

further shows that the choline and the symmetric dimethyl-arginine (DMA'), but partly the asymmetric dimethyl-arginine (DMA) and the monomethyl-arginine (MMA) too may partly cover each other. In the samples BT and BM not a single component could be identified with this method.

Results of layer chromatographic examinations. New possibilities of studying amino acids opened with the use of chromatoplates coated with resin (DÉVÉNYI—ZOLTÁN 1970), which we endeavoured to make use of by elaborating a method suitable for our purposes. Fig. 2 shows the separation of the 29 amino acids so far identified in the proteins. Accordingly, the basic protein amino acids and their derivatives can be readily separated by this method both from one another and from the other amino acids. In Fig. 3 we can see in a similar system (buffer 1.) the basic amino acids and their derivatives with BT samples, as well as with partly desalted sample of urine from a healthy grown-up man. It can be seen from the figure that mainly in sample BT-II the presence of a considerable amount of guanidino-methylized arginine and ε-N-methylized lysine as well as other unknown substances can be supposed, but identification cannot be carried out in this way. Fig. 4 is an example of the identification of ε-N-trimethyl-lysine isolated during the preparative examination of sample BT-I on a Fixion layer. The same figure shows the picture of two heterogeneous fractions the components of which were further studied by adsorption (Fig. 5) and partition layer chromatographic methods (Fig. 6). In sample

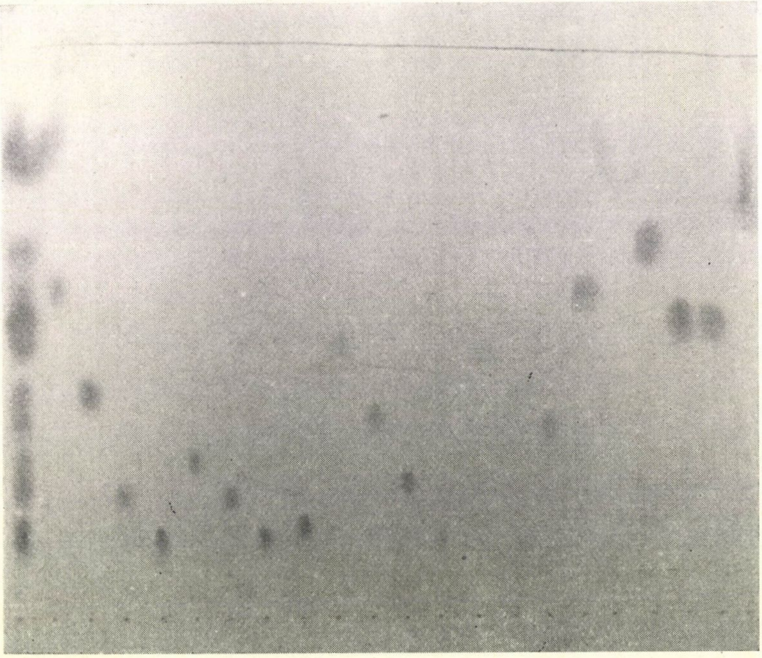


Fig. 2. Separation of amino acids occurring in peptide bond on Fixion-50 × 8 ion-exchange layer. Solvent: 2) buffer. Solvent distance: 16 cm; detection: ninhydrine. (1) Total test; 2—2 μg (2) lysine (3) MML (4) DML (5) TML (6) arginine (7) MMA (8) DMA (9) DMA' (10) histidine (11) 1-Me-His (12) 3-Me-His (13) L-tryptophane (14) L-tyrosine (15) L-proline (16) L-phenylalanine (17) L-methionine (18) L-treonine (19) L-valine (20) L-leucine (21) L-iso-leucine (22) 2—2 μg L-serine, L-alanine, L-glutamine, L-asparagine, L-glutamic acid, L-aspartic acid, L-cisteine, glycine

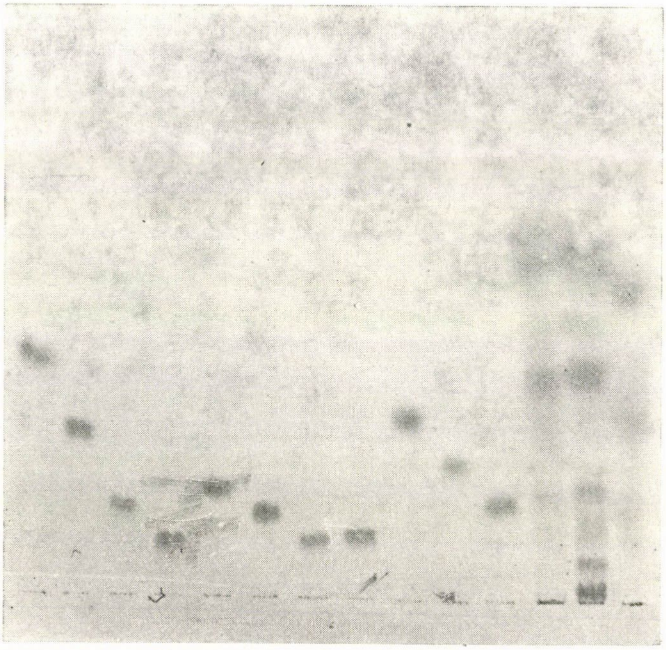


Fig. 3. Comparative study of basic amino acids, BT-samples and a urine sample on Fixion-50×8 layer 2—2 μg (1) lysine (2) MML (3) DML (4) TML (5) arginine (6) MMA (7) DMA (8) DMA' (9) histidine (10) 1-Me-His (11) 3-Me-His; (12) BT-I (13) BT-II (14) urine sample

Fig. 4. Chromatography of BT-I components isolated from Fixion-50×8 layer on Fixion-50×8 layer with authentic substances (1) "2" (2) "2"+TML (3) "2"+DMA' (4) TML (5) DMA' (6) "3" fr. (7) "4" fr. (8) MMA (9) DML (10) 3-Me-His

Fig. 5. Separation of basic amino acids by thin-layer chromatography on Silica Gel G layer. Solvent: chloroform-methanol-25% NH_3 (4 : 4 : 1). Detection: ninhydrin-reagent

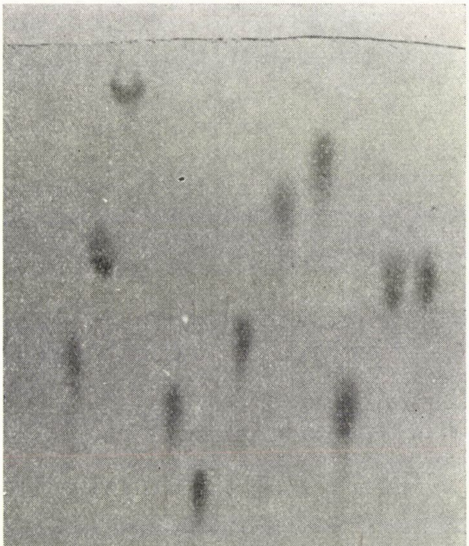


Fig. 6. Separation of basic amino acids by thin-layer chromatography on MN-cellulose powder 300 layer. Solvent: chloroform-methanol-25% NH_3 (4 : 4 : 1). Detection: ninhydrin-reagent (1) lysine (2) MML (3) DML (4) TML (5) arginine (6) MMA (7) DMA (8) DMA' (9) histidine (10) 1-Me-His (11) 3-Me-His

3 besides ε-N-trimethyl-lysine we succeeded in pointing out the presence of N^G, N^G-dimethyl-arginine (DMA), while in sample 4. only N^G,N^G-dimethyl-arginine was found to occur. Treating sample BT-II in a similar way N^G,N^G-dimethyl-arginine (DMA) could be identified. Studying samples BM-I and BM-II with the layer chromatographic method the same components could be identified as in the BT samples, only quantitative differences were found. Otherwise, in the case of identifications made with the adsorption- and partition-layer-chromatographic methods besides the ninhydrin reagent identification could also be carried out with the Dragendorff reagent on the basis of specific red and orange reactions.

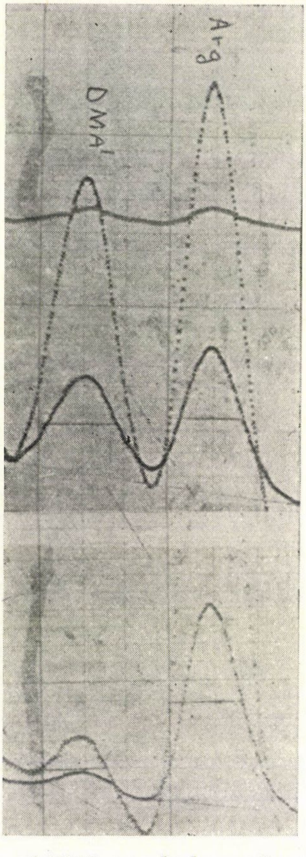


Fig. 7. Study of BT-I sample by amino acid analysator

Qualitative and quantitative studies by amino acid analysator. The lower part of Fig. 7 shows the result obtained by examining sample BT-I in an amino acid analysator. According to this beside arginine the sample contained a relatively large quantity of a dimethyl-arginine-like compound, and by adding authentic N^G,N^G-dimethyl-arginin (upper part of the figure) the respective peak increased. Considering, however, that under similar conditions the symmetric dimethyl-arginine (DMA′) left the column only one minute later, we can only say of the investigations made in the present system that compounds of dimethyl-arginine character (total dimethyl-arginine) were found in BT-I in an easily measurable quantity. Otherwise, in sample BT-I we succeeded in pointing out ε-N-methylated lysines, first of all ε-N-trimethyl-

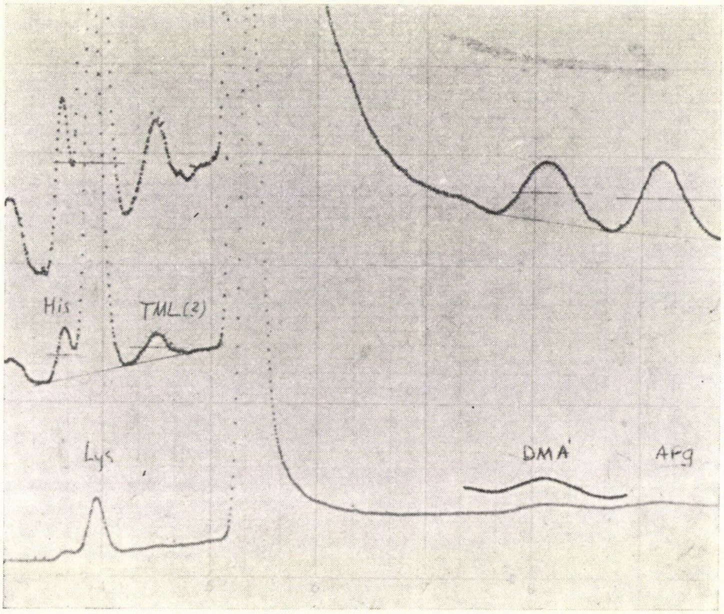


Fig. 8. Study of BMT-II sample by amino acid analysator

lysine in relatively low quantities compared to the lysine, but their perfect separation and precise identification have not been solved so far. Fig. 8 shows the basic amino acid range of amino acid analysis made with sample BT-II. According to this in the sample the quantity of dimethyl-arginines was nearly the same as that of arginine, and the results corresponded to those obtained in layer chromatographic studies. This figure further shows that the amount of total methylated lysines is significant. Fig. 9 presents the most important results of the amino acid analysis of sample BM and it can be seen that — besides the arginine — the quantity of dimethyl-arginines is also considerable though does not attain the values obtained with samples prepared in the same way from calf thymus.

Table 1 shows the proportions of basic amino acids and their derivatives found in samples BT-I and BT-II to lysine and to one another. The data show that perfect isolation with Reinecke salt of methylated basic amino acids was only successful in the refrigerator. Table 2 presents the quantitative conditions of basic amino acids and their derivatives as calculated for 100 g thymus gland. The most important conclusion of these data is that the amount of total dimethylarginine in the thymus of a healthy calf is considerably higher than that of the total methylated lysine.

Table 1

Proportions of basic amino acids in preparations obtained from calf thymus to lysine and to each other

Samples	His	Lys	Me$^{\varepsilon}$-Lys (total)	Arg	DMA (total)	Arg/DMA
BT-I	0.26	1.0	in traces	0.87	0.079	11.0
BT-II	0.11	1.0	0.074	0.48	0.42	1.14

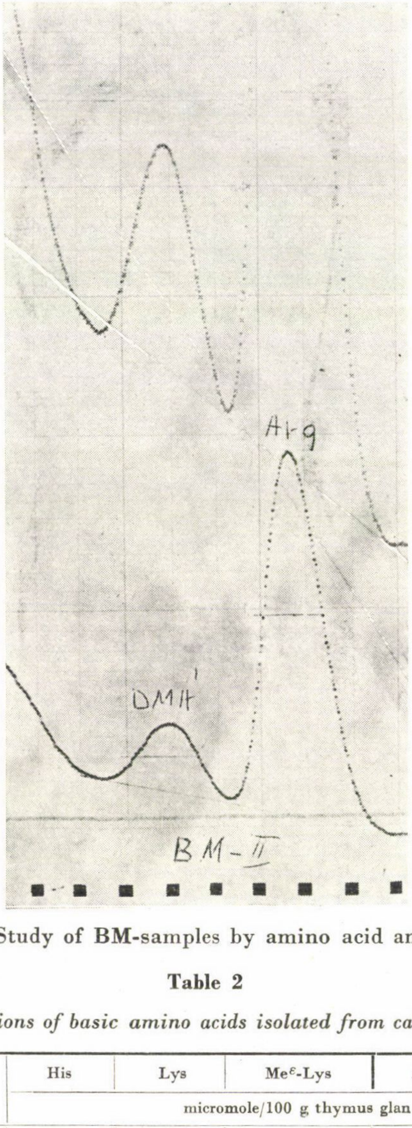


Fig. 9. Study of **BM**-samples by amino acid analysator

Table 2

Quantitative relations of basic amino acids isolated from calf thymus gland

Sample	His	Lys	Me$^{\varepsilon}$-Lys	Arg	DMA
	micromole/100 g thymus glan				
BT-I	1.28	4.86	0.06	4.26	0.38
BT-II	0.10	0.97	0.072	0.46	0.41
BT total	1.38	5.83	0.132	4.72	0.79

Discussion

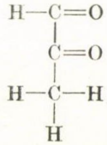
In the last few years ε-N-methylated derivatives of L-lysine and guanidino-methylated derivatives of L-arginine were identified in calf thymus histones (MURRAY 1964, PAIK—KIM 1967, HEMPEL *et al.* 1968, PAIK—KIM 1970), and even their wide occurrence both in a free and a bound form could be pointed out (summarized by Tyihák 1972). Paik—Kim (1968) found two enzyme systems to be present in calf thymus; one of them is the ε-NH_2 group of lysine in peptide-bond, while the other is able to methylate the guanidino group of arginine.

From the investigation results so far obtained their free occurrence could be expected in calf thymus and calf liver as decomposition products of histones or other proteins. With the method used by us we succeeded in enriching, in the form of reineckates, the methylated basic protein-amino acids, and so partly identifying them.

Using the paper chromatographic method employed by Szent-Györgyi *et al.* (1962) we could not identify a single component in preparations made from calf thymus and liver, and the uncertainty in determining the chemical nature of promine and retine is thus easy to understand.

Unfortunately, Szent-Györgyi *et al.* (1962) did not publish the reagents applied in their paper-chromatographic identifications, but from the remark made in a foot-note, as to choline and betaine having disturbed the chemical characterization of retine and promine we can conclude on the type of compounds and nature of reagents supposedly used. Since choline and betaine give no ninhydrine reaction, it is probable that the authors did not examine their preparations for amino acids. Choline and betaine are known to show more than one chromatographic reactions of which the Dragendorff-reaction is one of the most specific, that is why it was used in our comparative studies too (Fig. 1).

If the compounds found in calf thymus and calf liver, also studied by us in a free state, are to be compared with compounds determined for the structures of promine and retine, the problem arises that we can only present compounds for retine, as promine has only been mentioned so far, but its exact structure has never been described.



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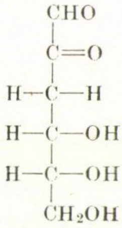

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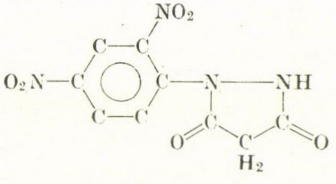

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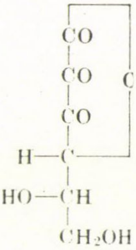

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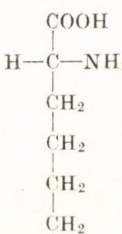

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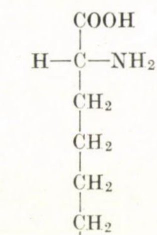

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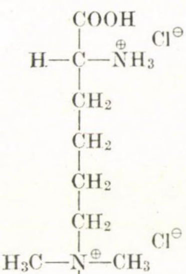

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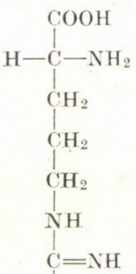

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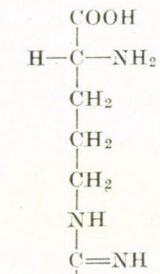

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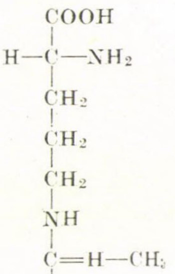

X

As compared to methyl-glyoxal (I) (Együd 1965), 3-deoxy-D-glucosulose (II) (Fodor *et al.* 1967), "free" retine (III) (Marmasse 1966), dehydro-ascorbic acid (IV) (Edgar 1969) the ε-N-methylated lysines (MML (V), DML (VI), TML (VII)) and the guanidino-methylated arginines (MMA (VIII), DMA (IX), DMA' (X)) satisfy the original criteria of promine-retine, namely, in a highly acidic medium form reineckate, contain nitrogen, are of highly similar structure, of low molecular weight; arginine itself, but more so its guanidino-methylated derivatives are easy to decompose, contain an unstable group and are more sensitive to alkalis than to acids.

As to the biological activity of the substances, Szende *et al.* (1970) were the first to point out the significant tumour growth promoting effect of DL-N^{ε}-trimethyl-lysine, and today extensive studies are carried on with this compound of specific activity by the above work collective (Kopper *et al.* 1971). The other two ε-N-methylated lysines have a similar stimulating effect on tumour growth (Annual report of the Institute of Pathological Anatomy, 1971). DL-N^{ε}-dimethyl-lysine promoted the growth of tobacco tissues to a considerable extent too, together with an essential change in the histone spectre (Tyihák *et al.* 1971). The retine character of guanidino-methylated arginine derivatives will be unambiguously decided by the current biological investigations.

On the basis of earlier experimental results it was supposed (Tyihák 1972), and now, after investigations made with calf thymus and calf liver, it is stated that it was these amino acid derivatives occurring in traces but biologically active that Szent-Györgyi *et al.* (1962, 1963) isolated in their original work, however, they did so together with impurities.

The investigations made with methyl-glyoxal- or glyoxal derivatives may be an important contribution to solving the problem of cancer, but in the original work much more was at stake; it was about the existence in normal tissues of such substances as being in relation with growth in general, and with fertility and even muscles too. We only add now that the ε-N methylated lysines and methyl-arginines have recently also been found e.g. in myosins and actins of the most diversified origin (Huszár—Elzinga 1969, Hardy *et al.* 1970, Reporter—Corbin 1971). Hegyeli *et al.* (1963) found retine in human urine too, but could not identify it either. It is very interesting that relatively large quantities of ε-N-methylated lysines and methyl-arginines have recently been pointed out in the human urine too (Asatoor 1969, Tyihák 1969, Kakimoto—Akazawa 1970).

Summing up we can say that the ε-N-methylated lysines identified with "promine" and the guanidino-methylated arginines identified with "retine" are common products of the living including the human organism, which, as yet, have mostly unknown functions, therefore the study of their formation and occurrence in free and bound forms offers immense possibilities to therapy as well.

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CONTRIBUTIONS TO THE PAPER OF M. KECSKÉS, J. M. VINCENT: "COMPATIBILITY OF FUNGICIDE TREATMENT AND RHIZOBIUM INOCULATION OF VETCH SEED" PUBLISHED IN THIS PERIODICAL, *22* (1—2)

WHAT EFFECT DOES THIRAM HAVE ON THE NODULATION OF VETCH PLANTS UNDER DIFFERENT SOIL AND CLIMATIC CONDITIONS?

The aim of the paper under discussion is to test the compatibility of the fungicide treatment of legume seeds and nodulation in the case of *Vicia faba*. Unconventionally, this paper does not consist of special chapters concerning methods, experiments and discussion. On the contrary, the authors state their results as a hierarchy of experiments starting with simple in vitro-tests and finishing with field trials. This method, I find, is indeed a possible one, but no doubt it renders the reading more difficult.

The literature discussed is arranged in three periods (1926—41, 1941—58, 1958 up to now). This division in three phases may be justified, however, it would have been useful to mention the causes of this phenomenon produced by changes of the fungicide spectrum. The list of the papers cited, of course, never can be complete, but the publications of DIATLOFF (1970) from Australia, GILLBERG (1971) from Sweden and some other authors should have been mentioned.

The experiments having been made during a stay of the first author in Australia are reported and discussed in the following sequence:

1. The inhibition of the growth of Rhizobium leguminosarum on agar plates by fungicide-dressed seeds and fungicide-treated filter disks.
2. The survival of *Rhizobium* on fungicide dressed seeds.
3. The nodulation of vetch plants having developed out of treated seeds and growing on seedling agar.
4. The nodulation of vetch plants having developed out of dressed seeds in sand culture.
5. The nodulation in *Rhizobium*-deficient soils after seed-dressing, partly with and partly without inoculation in a green-house test.
6. The nodulation in a field trial.

The following 7 preparations were tested: Panogen and Ceresan containing Hg as an active ingredient, Cuprox containing Cu, Captan, Thiram and the quinones Phygon and Spergon.

The results obtained show:

1. It is only the seed-treatment with Thiram that does not diminish the nodulation of vetch plants, the application of all the other preparations declines the development of nodules.
2. Out of the several tests only the study of the survival of *Rhizobium* on treated seeds and the green-house experiments agree in their results with the most important assay—the field trial.
3. The fungitoxic effect of Thiram assayed by inhibiting the root rot pathogen Thanatephorus cucumeris (Frank) Donk renders very well.
4. The nodulation occurring only at the lateral roots is a sign of reduced inoculation by Rhizobium.

In their paper the authors clearly state their results. However the question is, why they have described in detail the in vitro-tests that are of little interest in the evaluation of the real compatibility of the fungicide-treatment of legume seeds and their nodulation. A short summary of the most important results would have been sufficient for understanding the quintessence of the tests compared.

The publication under discussion contains some mistakes and misprints. Thus the name of the pathogen fungus should have been written "Thanatephorus cucumeris" and the statement of the toxicity ratios (p. 6) is not correct either.

On the whole, the text concerning the in vitro-tests is too long. However we can thank the authors for their successful efforts to find in Thiram a very useful fungitoxic preparation for treating vetch seeds against fungal pathogens without damage to the nodulation of this plant. However, it seems to be necessary, to confirm this important result under other soil and climate conditions.

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HOW DO LOSSES IN NODULATION COMPARE WITH REDUCTION IN HEALTHY SEEDLINGS FROM AN ECONOMICAL ASPECT?

Seed protectants are applied primarily to protect seedlings from infection by seed- or soil-borne pathogens. For this, a high fungicidal potency is required. Side-effects of seed protectants are usually limited to those cases where seeds are simultaneously inoculated with organisms essential for the development of crop plants.

The paper under discussion deals with the exceptional case of a coincidence in space and time between Rhizobia and seed. Based on the occurrence of negative side-effects, the authors have arranged seed protectans in an order of suitability, with Thiram as the least toxic chemical. In doing this, it should not be overlooked that the primary function of Thiram, as mentioned above, is as a seed protectant. Unfortunately, the very important economical aspect of weighing losses in nodulation against reduction in healthy seedlings has not been considered by the authors. In vitro experiments with the soil pathogen *Thanatephorus* (not *Thanetophorus!*) *cucumeris* offer no compensation for this shortcoming.

When using pure laboratory techniques for the evaluation of toxic effects, particularly in combination with nutrient media, better use should be made of the present state of knowledge. Numerous effects of fungicides can be derived from their physical and chemical properties.

It must be further pointed out, that simply citing 56 pertinent publications, without discussion of how the present work is related to those studies, seems to be inconsistent with the purpose of exchanging ideas and comments in the "Forum".

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CAN MERCURIAL SEED DRESSERS BE EXCLUDED FROM THE SEED TREATMENT OF CULTIVATED PAPILIONACEAE OWING TO THEIR GERM DAMAGING AND MICROBICIDE EFFECT?

The paper extending to 25 typewritten pages with 11 tables, published in the Forum column deals with an important subject, as it wishes to give an answer to the question: what are the direct and indirect effects of the seed treatment of cultivated plants belonging to the family *Fabaceae* with fuagicides on the *Rhizobium leguminosarum* species promoting the development of these plants. The experiments were performed in 3 phases, partly in the form of laboratory tests, partly as *Rhizobium* inoculation glasshouse- and light chamber- and outdoor small-plot experiments, respectively, using *Vicia sativa* seeds treated with fungicides. The results were evaluated on the basis of the dry-matter weight of samples taken from 12 and 18 weeks old plant stands, and by the analysis of root nodule forms described by the authors. The harmful or stimulating effects of fungicides on both inoculated and control samples were studied.

It was not surprising that of the fungicides used for seed treatment first of all the mercurial preparations (e.g. Ceresan) and the highly agressive Spergon decreased significantly the effect exerted on the rhizobium inoculation, while at the same time Thiram containing TMTD active ingredient increased it.

Captan, containing phtalimide immediately followed Thiram in its favourable effect, proving good, sometimes even better than the latter — e.g. in the B-serie of experiments — especially in the laboratory experiments. The results were always evaluated by the presence or absence of the root nodules, by their number and distribution, and on the basis of the patterns of root nodule formation, and it was found that Thiram and Captan had no harmful effects on the rhizobia, moreover, sometimes even a certain stimulative effect could be felt. Of the mercurial chemicals panogen is an unstable fungicide, as in the case of seed treatment after some time its microbicide effect decreases considerably. Preparations of copper content (Cuprox) showed moderately inhibiting or destroying microbicide effects. Likewise, it would have been important to try the activity of the zineb and maneb type fungicides applied for grain immersing.

It is regrettable that the Captan-like preparations (e.g. Orthocid) were not given proper emphasis in field treatments after the preliminary laboratory- and glasshouse experiments, since they are supposed to be — beside TMTD — similarly favourable and stimulating, or at the worst indifferent fungicides, which may be used for the seed treatment and simultaneous rhizobium inoculation of vetches and other cultivated papilionaceae beside Thiram. This fact should be all the more so emphasized as in Hungary TMTD and Captan are permitted to be used at a rate of 2—3 g/kg for the seed dressing of vetches, beans, peas, lentil, lupine and other cultivated papilionaceae.

Fortunately, the phytotoxic mercurial seed dressing preparations, which have a germ damaging effect on the seed of cultivated papilionaceae, have also affected — due to their high microbicide action — the rhizobium inoculations unfavourably and thus can be completely excluded from the seed treatment of the cultivated papilionaceae (e.g. alfalfa and clover). They have to be replaced everywhere first by TMTD and in the second place by preparations of Captan content. According to the results of experiments it is mainly TMTD that can be recommended for a joint application with rhizobium inoculation.

The authors' experiments were conducted over several years with great circumspection in a wide field. The experiments were planned at an up-to-date level and well thought over. The fungicides used were properly chosen; we might just add that if the experiments were continued, fungicides containing benomyl (benlate) ought to be included besides the TMTD and Captan active ingredients. A great merit of the authors' work is that the evaluation of the fungicides was performed on sound bases, since on the grounds of the various patterns of root nodule formation they elaborated a uniform system of evaluation.

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WHAT IS THE CONNECTION BETWEEN THE LITERATURE USED AND THE OBTAINED RESULTS?

The problem discussed in the work by M. Kecskés and J. M. Vincent is very interesting for both: science and agricultural practice, as can be seen from the list of numerous bibliography at the end of the work.

It is my opinion that the work would have gained in its value if the basic research problems of the different periods had been given in the historical review of the literature.

In this study the effects of six organic and an inorganic fungicide (Panogen, Ceresan, Cuprox, Thiram, Captan, Phygon and Spergon) on seven strains of *Rh. leguminosarum* sp. and their nodulation with *Vicia sativa* L. were examined. The experiments were carried out in laboratory, lighted room, glasshouse and in the field. The applied methods as well as the evaluation of the results were correct and modern, quite at the level of present day science.

The fungicide treated seed and fungicide impregnated filter discs showed consistent and large differences among fungicides when the method of inhibition zones was applied. The most toxic fungicide was Panogen. The other Mercurial, Ceresan was the second potent. Cuprox, Thiram, and Captan comprised the intermediate group. The least potent were Phygon and Spergon.

In the case of Phygon the growth of most of the strains was delayed but not altogether prevented.

Of all the examined fungicides Panogen was the most unstable, especially when stored in a lighted place over a long period of time.

The examination of the vetch plants' nodulation on both seedling agar and washed river sand showed very similar results.

Ceresan completely prevented or caused 46 percent delayed nodulation. Thiram and Captan were the least inhibitory, while Spergon, Phygon and Panogen showed intermediate inhibition.

The occurrence and distribution of nodules were carefully evaluated in the glasshouse experiments with Thizobium deficient soils.

An early nodulation (78—85 percent) on tap roots, which were inoculated but were not fungicide treated was indicated in all the three types of soil. With all the fungicides except Thiram the number of tap root nodulated plants was significantly lower than with the control. Panogen and Ceresan were the most incompatible of the examined fungicides. Ceresan, Phygon and Spergon caused considerable delays.

The delay in nodulation caused by Ceresan varied on the Lismore soil. The field trial results agreed with the results obtained under glasshouse conditions. The location of the nodules and their number indicated the incompatibility of Ceresan and Spergon as opposed to Thiram. A very important result of the research was that the percentage of crown plants is in correlation with the yield.

This work gave the answer on the compatibility between the rhizobium inoculation of vetch and the fungicide treatment. Thiram was found to be the most compatible fungicide. This work also has methodical value because it evaluates different methodical value because it evaluates different methodical treatments.

The work deserves considerable attention and will be found interesting for both scientists working in this area and agronomist practicioners. However, the work would have been more valuable if the literature used in the work had been better connected with the obtained results which would enable a reader to judge for himself on the novelties in the work as well as the problems which were faced in the course of the experimentation.

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WHAT ARE THE DIFFERENT RESPONSES OF RHIZOBIUM TO INDIVIDUAL FUNGICIDES IN DIFFERENT SOILS?

This paper deals with very important topics on the relationships between the fungicides in present agricultural use and *Rhizobium leguminosarum* as an organism causing the nodulation of vetch roots.

The introductory brief historical review gives the necessary understanding about the development of chemicals applied in plant protection in the last half a century and the microbial aspects of their effects.

The six fungicides examined in the present study represented the most important groups of fungicides used in agriculture today.

The experimental scheme is clear and enables the good comparison of the effect of individual treatments.

It has been demonstrated that there are big differences in the compatibility of nodule microorganisms to individual fungicides. In general, Ceresan had the most harmful effect on plant root nodulation whereas Thiram was the least injurious. The biological tests yielded almost the same results whereas the laboratory agar plate test did not give satisfactory responses.

The effect of the soil, where the plants are grown, is also of distinct importance. It is regrettable that the authors did not explain the different responses of *Rhizobium* to individual fungicides in different soils.

The increased dry top weight of inoculated and Thiram-treated plants in comparison with those just inoculated as well as the yield depression after the application of Ceresam or Spergon allows further conclusions on the mutual effect of *Rhizobium* and fungal parasites on vetch. The authors did not make full use of this opportunity.

Summarizing, it could be said that this paper gives very important information on the possibility of simultaneous Rhizobium inoculation and the fungicide treatment of vetch seed. There is a great need for these data at present.

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WHAT METHODS SHOULD BE USED FOR THE FUNGICIDE TREATMENT AND INOCULATION OF LEGUMINOUS PLANTS?

In this paper the authors give the results and their carefully made interpetations of a long and original investigation about the compatibility of fungicide treatment and rhizobium inoculation. This problem is very important not only from a theoretical but also from a practical point of view. The inhibitory effect of pesticides on plants, soil microorganisms, animals and human beings is a basic problem today. The investigation of Dr. Kecskés and Dr. Vincent on this basis gives a practical contribution to the work of modern agriculture.

The way the authors state the problem and elaborate it shows their competency and erudition. The long list of cited literature — with more than 50 titles makes a very good impression.

The authors investigated six organic and one inorganic fungicide for the treatment of vetch seed inoculated by seven strains of *Rhizobium leguminosarum* sp. Frank. They also investigated their nodulation with vetch *(Vicia sativa* L.). The authors performed precise experiments using different methods, e.g. the agar diffusion method, the determination of the survival of rhizobia on inoculated seed, the nodulation of vetch plants on seedling agar, in washed river sand and in soil and also the determination of the plant yields in field trials. Using so many different methods the authors have succeeded in gathering a big quantity of data of different points of view. This has enabled them to draw a correct conclusion about the compatibility of fungicides with inoculation with rhizobia.

The results are shown in 11 tables. They give a very good and detailed illustration of the investigation. They show the superiority of Thiram over all other fungicides used in the experiment. The data of the agar diffusion method detect some markedly incompatible substances, e.g. Panogen and Ceresan. The data from the different trials correspond very well with each other. E.g. the data of the glasshouse trials correlate very well with the final criterion — the field trial. This allows in a highest degree the authors to make correct conclusions.

The investigation of Dr. Kecskés and Dr. Vincent is very useful for agriculture. It is important not only for the purpose of vetch cultivation but also for all leguminous plants subject for treatment by rhizobia as inoculum. It is necessary in the future that such investigations should by all means be done before undertaking the fungicide treatment and inoculation of leguminous seeds. The authors show methods for this purpose and some of them are simple, e.g. the agar diffusion method. It can be applied in every agricultural laboratory. It is not expensive and does not take a long time or require special equipment. The authors show that using filter discs is better than seeds treated by fungicides in this method. There are also other similar methods by using holes with fungicides in agar or rings of agar preliminarily treated by fungicides instead of using filter discs. They are also good and only special investigation may determine which of them is the best for a concrete purpose.

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WHAT ARE THE EFFECTS OF DIFFERENT FUNGICIDES ON RHIZOBIUM SP. AND NODULATION ON THE ROOTS OF LEGUMINOUS PLANTS?

Chemicalization in agriculture has led to the use of a great number of different pesticides (insecticides, fungicides, herbicides) for the control of pests, diseases and weeds in crops. Most frequently these chemicals are complex chemical substances with great physiological activity. This makes it necessary to clear up a lot of problems concerning their use — their action and effect, penetration and metabolism in plants, the residual quantities of these chemicals in the produce and so on.

During the last decade thorough investigations have been conducted in many countries for clarifying the problems related with the absorption, metabolism and detoxication of pesticides in the soil, as well as their influence on soil microflora and the intensity of microbiological processes. Of the various microorganisms in the soil, the subject of more extensive investigations in this direction were free living N-fixing bacteria of *Azotobacter* and *Clostridium pasteurianum*. In recent years investigations for establishing the effect of different pesticides on the bacteria of *Rhizobium* sp. and nodulation in the roots of leguminous plants have increased. A greater part of these investigations refer to the herbicides (CARLYLE—THORPE 1947; FLETCHER—SMITH 1964; CHETZALIN 1964; YORDAN—GARTZIA—GERARD 1966; NEPOMILUJEW—BEBIN—KUSJAKINA 1966; RANKOV—ELENKOV—SURLEKOV—VELEV 1966; HAMDI—TEUFIK 1969; RANKOV—ELENKOV 1970; HAUKE-PACEWICZOWA 1971; KECSKÉS—BORBÉLY—BORBÉLY—ELEK 1972).

A great interest for agricultural practice is the problem of the influence of fungicides on *Rhizobium* sp. and nodulation when the seeds of leguminous plants are treated with them. This investigation is devoted to this problem. The work also summarizes some other investigations of the authors on the problem (KECSKÉS—VINCENT 1969, KECSKÉS 1970).

After a profound review of the problem, in 8 sections, the authors discuss the effect of different fungicides on strains of *Rh. leguminosarum* under laboratory conditions and nodulation on vetch roots *(Vicia sativa)* in vessel experiments in the glasshouse and under field conditions. So in the conducted laboratory and vessel experiments (section I to VI) the effect of the fungicides tested on strains of *Rh. leguminosarum* is studied — Inhibition zone on seeded agar plates and with impregnated filter discs; Influence of fungicide on the survival of rhizobia on the inoculated seed; Nodulation of vetch plants on seedling agar; Nodulation in washed river sand and nodulation in *Rhizobium* deficient soils — glasshouse experiment, carried out on 3 different types of soil, using vetch *(V. sativa)* as test-plant in these cases.

Section VII gives the results under field conditions, while section VIII gives a conclusion of the results obtained. In the experiments with nodulation in vessels or under field conditions the presence or abscence of nodules, their number and distribution on the root system is noted.

The influence of 7 fungicides — Panogen, Ceresan, Cuprox, Thiram, Captan, Phygon and Spergon is studied.

In the investigation different treatment combinations are used — with or without fungicide, and with bacteria.

The results obtained are well illustrated in 11 tables. The different toxicity of the fungicides depending on the chemical composition, length of treatment and storage conditions are emphasized.

Of further interest is the investigation on the effect of these fungicides on the development and productivity of vetch *(V. sativa)* and plant responses. For instance, the investigation carried out under field conditions during the growing season with the herbicide monolinuron indicated that initially the herbicide depressed nodulation on bean roots *(Phaseolus vulgaris)* (ELENKOV—SURLEKOV—RANKOV—VELEV 1970). This depression was short-term after which there was an initially low, but after that high stimulation effect on nodulation. This led to an increase of not only the above-ground material, but also of the yield of the bean (9.5% higher). It is also known, that the biological fixation of nitrogen from nodule bacteria is connected with the symbiosis between them and leguminous plants. The other investigation, carried out with the herbicide Trifluralin during the growing season of 11 bean varieties *(Phaseolus vulgaris)* showed that the influence of the herbicide on the root nodulation of different varieties is different (RANKOV—ELENKOV 1970). The changes in nodulation in this case are explained with the response of the varieties.

Finally we have to note, that the great experimental work gives an opportunity to estimate the effect of the fungicides included in the investigation on *Rh. leguminosarum* and nodulation on vetch roots *(V. sativa).*

The investigations are also interesting from a methodical aspect. Undoubtedly this investigation will contribute to the broadening of the knowledge in the sphere of modern theory and practice on the problem. We can expect positive appraisal and grat interest from the readers — specialists in this sphere.

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CAN THE NODULATION BIOASSAY OF PAPILIONACEAE BE USED FOR TESTING THE LEVEL OF PESTICIDE (FUNGICIDE) RESIDUES IN THE SOIL?

The nodulation bioassay elaborated by the authors provides data easy to evaluate for the comparison of the damaging effects caused by fungicides (in general pesticides). Generalizing the obtained results, the influence of pesticides on the microbial activity of the soil can be reasonably characterized by the nitrogen fixation of rhizobia and the inhibition of nodulation. The intensity of nitrogen fixation, and inhibition of metabolism in the nodulation organisms provide direct quantitative data for finding a correlation with the level of chemical residues in the soil, further, the different special fungicides could be classified by their toxic effect on soil-borne microorganisms. Especially this latter state of affairs offers a possibility for comparing the degree of compatibility of the nodulation organisms, evaluate the biological activity of agent groups in relation to the application of different chemicals on the basis of a qualification of side-effects.

Correlation between nodulation inhibition and pesticide-like chemical residues (metabolic inter-products) is a multifactorial correlation in the development of which synergic effects may play a role too, still it is almost the only possibility of microbial soil testing, since the qualification of biological effectivity is much more sensitive than the microchemical identification. The extent of soil contamination is not a negligible question from the point of view of environmental protection either, and the multifactorial correlations too can only be interpreted by a biological data survey.

Qualification of differences in compatibility between the nodulation organisms (from incompatibility to a high degree compatibility) as well as interpretation of relations with the metabolic processes, when based on an extensive fungicide testing, may provide highly valuable information for the study of multifactorial correlations.

Subsequently the question of natural selection in the Rhizobium strains is raised on theoretical and practical levels, first of all in relation with resistance to pesticides widely employed in farm practice. It is highly probable that under the influence of an increased fungicide application in the last several years, strains possessing some resistance compared to Rhizobium strains living in symbiosis with the vegetation components of native grass fields have already been selected. Consequently it is supposed that on synthetic culture media general and special fungicide tolerance in the nodulation organisms can be increased by the application of fungicides, and that — by a lucky recognition of the exceedingly low number of inductions — the extreme types of fungicide resistance appear too. The latter on the other hand are of very great importance in the corrected increase of soil productivity.

The high number of fungicides used in practice is expected to increase still further in the near future, and the nodulation bioassay elaborated by the authors will play an important role in determining the compatibility levels through in vitro cultures and field experiments alike. The identification of chemicals showing an especially high toxicity as well as a systematic planning of fungicide rotation offer more concrete possibilities for considering the application of pesticides. The characterization of pesticides by nodulation organisms provides — with the agent groups, compound types and levels — data obtained by new objective methods for the realization of environmental protection, through an integrated view of plant protection.

The study of metabolic processes, biochemical mechanisms and bioenergetic equations of nodulation organisms, supposedly first in relation with the compatibility degrets, is a highly important subject. The publications of the most recent years reveal that the nodulation organisms are able to reduce the molecular nitrogen (N_2) directly, by means of reduced NAD and NADP coferments, through proton transfer processes. The direct or indirect role played in the reduction by the haem type compounds which occur in outstandingly large numbers and high concentrations in the rhizobia is not known. The fundamentally biochemical process in the nitrogen fixation takes place in three steps, or else, three different mechanisms quite independent of one another are supposed to be involved:

$$1/2\ N_2 + 2\ H^+ \rightarrow -NH_2$$
$$1/2\ N_2 + 3\ H^+ \rightarrow NH_3$$
$$1/2\ N_2 + 4\ H^+ \rightarrow -NH_4$$

On the other hand, it is already quite certain that the continuous reduction of NAD and NADP may originate from either photosynthetic or oxidative phosphorylation. The symbiosis of nodular organisms with plants suggests that they take more advantage of the received reduced coferment system. At the same time, the nodular organisms are able to survive for a long time even without *Papilionaceae*, so they are supposed to be able to cover the metabolic reduction at the expense of oxidative cell respiration, probably with a very low intensity.

A turn over level determination of the absolute and relative intensity values of the nitrogen fixing reaction originating from the two different bioenergetic processes, in relation with the biological effectivity of various toxicity pesticides, is expected to provide more concrete possibilities for the interpretation of compatibility and/or fungicide resistance in the nodular organisms. Studies on the selective inhibition of biochemical mechanisms and bioenergetic processes not only lead to the recognition of biological effectivity in the special agent groups but also explain the activity of the nodulation organisms in the soil even in relation to the multifactorial interactions.

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WHAT IS THE BEST WAY FOR TESTING THE COMPATIBILITY OF FUNGICIDE TREATMENT AND RHIZOBIA INOCULATION OF LEGUMINOUS PLANTS?

Kecskés and Vincent in their article concerning the compatibility of fungicide treatment and rhizobia inoculation of leguminous plants speak about a very actual problem. It is of great importance to know what is the possibility of using seed inoculants together with pesticides, particularly with fungicides.

The work of Kecskés and Vincent concerned the effect of seven different fungicides on seven strains of *Rhizobium leguminosarum* Frank and their symbiosis with vetch. The experiments were carried out in a laboratory and under field conditions.

In conclusion the superiority of Thiram over all other fungicides was recognized as evident. On the other hand Ceresan appeared very toxic to Rhizobium. The differences between these two fungicides were beyond any doubt. The other fungicides intermediate in effect, were indistinguishable.

The laboratory tests were made with only one dose of each fungicide according to the manufacturers directions. Our earlier studies Golebiowska—Kaszubiak (1965) proved that different strains of single species of *Rhizobium* differ markedly in their reaction to fungicides. For example Thiram was inhibitory to different strains of *Rh. leguminosarum* on pea in a range from 100 to 600 μg/ml of liquid medium and phenylmercuric acetate respectively from 10 to 30 μg/ml. The similar differences among the strains of other species of *Rhizobium* were observed too. It is possible that by testing numerous doses of fungicides, and using more strains of rhizobia, the experiments could be more detailed and the differential inhibition of fungicides intermediate in effect could be determined more earily.

Comparing plant tests the authors talked about the superiority of the soil core method. Good repeated results were recorded under the employed conditions but Rhizobia — deficient soils were used for the tests and the local strains of Rhizobia, different in their reaction to different fungicides could not make these results out of order. Thus taking into account the local strains of Rhizobia the superiority of the soil core method compared with the seedling agar and sand culture methods could be in considerable doubt.

Though the plant tests, particularly with soil cores, in some cases can be more adequate to the final criterion — the field experiments — it seems that the repeated results and good correlation with the compatibility of different fungicides in agricultural practice may depend on local conditions. For this reason the different laboratory tests, as more fast and more detailed checks should give full weight in testing the compatibility of *Rhizobium* inoculation of leguminous plants and fungicide treatment in agricultural practice.

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REFERENCE

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DO THE DIFFERENT SPECIES AND STRAINS OF RHIZOBIA VARY IN THEIR SENSITIVITY TOWARDS FUNGICIDES?

In laboratory, lightroom and glasshouse experiments, carried out in the Microbiology Department of the Agricultural Faculty of the University of Sydney, the effect of six organic and one inorganic fungicide belonging to five different groups was investigated on seven strains of *Rhizobium leguminosarum* sp. Frank and their nodulation with *Vicia sativa* L.

Seed of vetch treated with the seven fungicides according to the manufacturer's direction was placed on plates of yeast extract- mannitol agar that had been seeded with *R. legumi-*

nosarum. From the inhibition zones it could be concluded that all fungicides were in some degree inhibitory: Panogen the most, Phygon and Spergon the least. However, the agreement between replicates was not good. Seed which had been treated seven months earlier was as inhibitory as the one-month old seed used in the first experiment in the case of 6 of the fungicides, but the activity of Panogen had been considerably reduced during this longer period of storage. It was shown that this fungicide is unstable, rather more so when illuminated.

Experiments carried out with impregnated filter discs showed consistent and large differences between fungicides: Panogen was the most toxic, followed by the other mercurial, Ceresan, an intermediate group of aprroximately equal potency (Cuprox, Thiram and Captan) and again the least potent, the quinones: Phygon and Spergon. Similar tests with the seedling pathogen *Thanetophorus cucumeris* followed the same order, with the exception of copper oxychloride (Cuprox) which was the least toxic. Toxicity ratios between inhibition zones of *Thanetophorus* and Rhizobium show that Ceresan and Thiram were relatively less toxic towards the rhizobia than towards the patho en. Naturally it is not sure that this holds for all the other seedling pathogens of *Vicia sativa.* BRAKEL (1963) showed that different species and strains of Rhizobia vary in their sensitivity towards fungicides. However, nothing is mentioned in this respect for the seven strains used in the study of Kecskés and Vincent.

Survival of rhizobia on the variously treated seed that had been inoculated with a peat culture of *R. leguminosarum* determined immediately after application and after 12 and 24 hours was uniformly good in the case of Thiram and poor in that of Ceresan. The remaining fungicides were intermediate in effect.

Plants raised from inoculated fungicide and non-fungicide treated seed on agar were examined for presence or absence of nodules. Under these conditions Ceresan completely prevented nodulation, Thiram was almost without effect and the Spergon treated seedlings were generally well nodulated. Th3 other fungicides except Cuprox were intermediate in effect, whereas in the case of Panogen seed that had been treated 14 weeks or more before inoculation yielded more positive plants (81%) than that had been treated earlier (9%).

Two preliminary experiments were conducted in washed river sand (bottle jar assembly) with the 7 fungicides, the inoculated seed being sown 3—4 hours after inoculation. Plants were harvested in the sixth week and examined for presence or absence of nodules. Phygon and Spergon depressed nodulation on both occasions. Thiram appeared to be the least inhibitory. In a more detailed experiment with the same assembly, the distribution as well as the presence or absence of nodules was recorded. From this experiment the fungicides could be classified in order of decreasing compatibility: Captan, Thiram > Spergon > Phygon > Panogen > Cuprox > Ceresan.

The influence of the fungicides on nodulation was also studied in undisturbed cores of soil taken from three fields Sydney soil, Lismore soil and Narrabri soil) deficient in *R. leguminosarum.* Seed that had been treated with the range of fungicides and inoculated with a standard peat culture was sown on three occasions after inoculation (0, 6 and 24 hr.) and kept in the glasshouse. The presence of nodules was examined after 6 weeks, by which time all of the inoculated control plants were well nodulated. In all three soils the inoculated non-fungicide treated control plants showed evidence of early nodulation (78—85% on the tap-root, the remainder on lateral roots near the crown.) All uninoculated controls were free of nodules. Under these conditions all of the fungicides, except Thiram, departed markedly from the norm in that the proportion of early taproot nodulated plants was markedly less than the non-fungicide control. Captan, although markedly inferior to Thiram in these tests was significantly better than the other fungicides. The mean percentage of plants with nodulation on tap-root and on lateral roots only near the crown in the three soils for the non-fungicide control and for the treatments with Panogen, Ceresan, Cuprox, Thiram, Captan, Phygon and Spergon was: 100, 69, 40, 74, 75, 87, 53 and 56, respectively. Taking this percentage as indicative of speedy nodulation Captan proved to be superior to Thiram. In the last sand culture experiment as well as in the soil cores Thiram and Captan proved to be superior to the other fungicides. It is a pity, therefore, that Captan was not tested in the field experiment.

A field experiment was conducted on Lismore soil poor in *R. leguminosarum*. Seed of *Vicia sativa* was treated with Ceresan, Spergon or Thiram inoculated with commercial peat inoculum. The seeds were sown immediately and after 6 and 24 hours. A total of 240 plants (for each fungicide-inoculum treatment) were sampled in July and in September. The influence of fungicide on pattern and incidence of nodulation in July showed that the highest proportion of tap root nodulation were found in the untreated inoculated and in the Thiram treated plants. The percentage of nodules on the distal lateral roots, however, were much higher on plants treated with Ceresan and Spergon. There were a few nodulated plants in the uninoculated controls, but these were all restricted to the distal lateral roots. By the time of the second sampling about 30% of the uninoculated plants had become distally nodulated. For this reason the proportion of crown nodulated plants which is a good indicator of speedy nodulation was used to compare the effect of the fungicides. The superiority of Thiram and the incompatibility of Ceresan and Spergon were clearly shown in this way. Details of results for the three times of sowing (0, 6 and 24 hr. after inoculation) have been omitted because, although significant differences were obtained, they revealed no trend with time. This is interesting, since McNew—Hofer (1942) showed that it was necessary for pea seed treated with Spergon to be sown within half an hour of inoculation, while Milthorpe (1945) found that this was necessary within two hours of inoculation.

The order of superiority of treatments judged by the percentage of crown nodulated plants (Thiram $\gg$ Nil Fungicide $\gg$ Spergon $>$ Ceresan) is reflected very precisely in the respective yields. Plants having the tap roots nodulated carried a large number of nodules and were the largest plants (11—15 the weight of non-nodulated plants and almost twice the height). Plants that were nodulated on lateral roots but near the crown also had many nodules and yielded well. Distal lateral nodulation on the other hand, indicative of late and sparce infection, was distinctly inferior and in the worst situation was little better than the non-nodulated plant.

The great merit of this work is that Kecskés and Vincent compared laboratory tests, lightroom and glasshouse experiments (plant tests in seedling agar, in sand watered with nutrient solution and in soil cores) with a field trial. The best correlated laboratory result was the per cent surviving rhizobia on the treated seed which clearly separated Thiram from the rest. Plant tests neither in seedling agar, nor in sand watered with nutrient solution were by themselves insufficient to screen off all less satisfactory chemicals. However, glasshouse trials with undisturbed soil cores were quite adequate and correlated very well with the final criterion—the field trial.

Another merit of this work is that the writers give a most valuable survey of the relevant literature. The more it is a pity that in this work they fail to compare their results with those of other workers who have tested the same fungicides for their effect on the symbiosis of rhizobium. In the last decade Thiram, for instance, has proved to be the least injurious for its effect on the symbiosis of rhizobium by Hofer—Crosier (1962), Wróbel (1963), Jakubisiak—Golebiowska (1963), Golebiowska (1965), Golebiowska—Kazubiak (1965).

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LET US AIM AT COMPLETING REFERENCES?

The paper looked over is undoubtedly interesting. It is dedicated to a problem which is rather interesting for a wide range of agronomists and it seems completely suitable for publishing.

As for the text I can only make a few remarks. When referring to the literary sources it is expedient to give summaries especially on the papers in Soil biochemistry issued in 1947 and 1971 by P. C. Kearney et al., F. Matsumura, A. G. Boush, D. Woodcock, etc.

Rather a lot of works on the question dealt with have been published in the Soviet Union. These are not mentioned at all by the authors.

A discussion part about the materials cited and conclusions must be joined to the paper.

The references should not only be completed, but also reduced, omitting the authors' unpublished papers as well as those of some other research workers. It is unnecessary to make foot-notes about them, because these materials are out of the readers' reach.

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WHAT IS THE MINIMUM CONCENTRATION OF EACH FUNGICIDE REQUIRED FOR OBTAINING FUNGISTATIC AND FUNGICIDE EFFECT ON TUBERCLE BACTERIA?

The manuscript, submitted to us by M. Kecskés and T. M. Vincent on "Compatibility of Fungicide Treatment and Rhizobium Inoculation of Vetch Seed" is of great interest, as it considers an important problem, not only from a scientific, but also from a practical point of view. As the authors mention, the study of the preparations for plant protection has an increasing significance, not only from the standpoint of their application in agriculture, but also for microbiology, plant physiology, and for the entire living world.

The objective of the authors has been achieved by means of various contemporary methods, beginning with those, conducted under laboratory conditions — on seeded agar plates and reaching vegetation glasshouse and field experiments. The system of their methods is described in previous publications. The study presented is the result of continuous work, the final achievements of which are being summarized and acquire a survey character. A comprehensive review of literature has been made, giving a full picture of the importance of the problem and the value of the study.

The merits of the study are undoubtful, but with the purpose of further clarification of some problems, we consider that certain investigations could be made which would bring more clearness.

Before all, we consider it necessary that a study should be made of the minimum concentration (mg/ml) of each fungicide, required for obtaining fungistatic and fungicide effect on different species of tubercle bacteria. This is necessary so that this concentration can be compared with the one applied in practice. A pure experiment should be conducted — with pure preparations and microbe strains in order to establish the mg activity of each fungicide. A comparison could be made between the fungicide effect on the vital ability and the development of strains of the species Rhizobium in using various working concentrations.

For establishing the minimum concentrations, acting fungistatically or fungicidewise on tubercle bacteria and the action on different amounts of fungicides, we recommend the diffusion method (with solid or semi-solid agar to which a certain amount of the fungicide is added) or the method of dilution in liquid nutrition media. These experiments could also determine the effect of the time factor on a contact between the fungicide and the microorganisms. The results obtained after these experiments have a great significance for the quantitative evaluation in carrying out the vegetation experiments, as they give information for the determination of the necessary outcoming fungicide concentration. We consider that the difference in the results of the experiments with filter disks, imbued with fungicides, is due actually to the inexact fungicide dosage.

Different fungicide concentrations could also be applied on peat inoculum and the following results be juxtaposed in reference to the survival and virulency of tubercle bacteria and nodulation activity.

In glasshouse experiments with undisturbed cores of soil the relationship between fungicide, soil, microorganisms, and plant could be elucidiated by additional experiments. The method consisted of planting seeds after their inoculation and fungicide treatment. We suggest applying different fungicide concentrations on the undisturbed cores of soil and then sowing the inoculated seeds. This experiment also helps in establishing the effect of soil type comparing the data for an equal quantity of fungicide in various soils and different amounts of fungicide in the same type of soil.

Evaluating the final results of the vegetative experiments for comparing differently treated plants, will further the completion of the investigations, moreover, if the amount of total and protein nitrogen is determined, it will help the quantitative juxtaposition of the changes in the activity of the tubercle bacteria.

These comments do not undervalue in the least the merits of the study of M. Kecskés, J. M. Vincent, but aim to draw the authors' attention to continuing the investigations on other sides of the problem for its further clarification.

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HOW SHOULD LABORATORY TESTS BE TRANSFERRED TO THE CONDITIONS OF A GIVEN FIELD?

The authors studied the important question of the relationship between leguminous plants, nodule bacteria and fungicides, a problem which jointly with the increasing intensification of plant production in general and the ever growing significance of leguminous plants for the humus-economy in particular, demands an unobjectionable elucidation. The extensive examinations comprised laboratory, green-house and field experiments. Results are given on the effects of Panogen, Ceresan, Cuprox, Thiram, Captan, Phygon and Spergon on seven Rhizobium strains and *Vicia sativa.* At the same time the experiments were designed so that the effects of fungicides could be examined on both the seeds and the nodule bacteria, furthermore on *Thanetophorus cucumeris*, the important seed-pest. The well designed and excellently performed experiments can be considered exemplary in this type of investigation. In these experiments a consistent procedure was followed, since at first fungicide-treated vetch seeds were brought-up on a *Rhizobium*-containing culture medium. From the here developing inhibition zone the efficiency of fungicides was read-off as usual. The strongest inhibition on *Rhizobium leguminosarum* was displayed by the fungicide Panogen. A time-effect was observed as six months after treatment the Panogen fungicide lost much of its effect. The pest *Thanetophorus cucumeris* was influenced in a similar way. A further comparison, however, showed that e.g. Ceresan and Thiram exhibited stronger toxical effects against *Thanetophorus cucumeris*, than against *Rhizobium*.

When inoculated vetch-seeds were treated with the above fungicides immediately after inoculation or 12 hours later there was a higher survival rate in *Rhizobium* with the Thiram treatment and a very low rate in the case of Ceresan. In all the laboratory experiments the toxicity degree of mercury-containing preparations was high against *Rhizobium*. Already in these experiments a favourable impact of Thiram is indicated, which exerts the least toxic effect on the nodule bacteria.

When the vetch plants were left to grow until the nodule formation, it was again with Ceresan that nodulation could be completely inhibited, whereas Thiram enabled an almost unhindered growth of the nodules. In experiments with quicksand Captan also proved favourable in this sense.

In *Rhizobium*-free soils the nodule formation of inoculated and fungicide-treated soils was likewise influenced by the preparations. A very insignificant nodule-formation on the lateral roots occurred with an application of Panogen, Ceresan, Cuprox, Phygon and Spergon. The formation of nodules was influenced only to a small extent by Thiram.

The results obtained in green-houses were far-reachingly confirmed by the field-experiments.

Summarizing, first of all the particularly positive attributes of Thiram can be confirmed. Without substantially impeding nodulation it is highly efficient against the pest *Thanetophorus cucumeris*.

The interesting comparison made here between the results of laboratory tests and field experiments points once more to the necessity that in the interpretation of results obtained in soil-biological experiments the effect of many individual factors should be taken in consideration, as well as an obligatory precaution in the transfer of laboratory tests to the conditions of a given field.

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IS IT ONLY THE EXTERNAL MORPHOLOGY OF ROOT NODULES OR THEIR INNER TISSUE STRUCTURE TOO THAT THE VARIOUS FUNGICIDES ACT ON?

Root nodule formation experiments performed in washed siliceous sand have shown a more intensive root nodule formation on the main roots of control plants than on the laterals. In the case of treatments with seven different fungicides both the number and place of the formed root nodules varied. This raises a number of organizational questions.

In the case of seeds treated with fungicides three hours before sowing the fungicides penetrate not only the surface, the seed-coat of the seed, but during the period of the treatment, or later on get inside the seed-coat to the embryo. To some extent the embryo takes up these fungicides which during the development of the seedling exert an influence on the root nodule formation of the main- and lateral roots. In the course of development this influence decreases and root nodule formation by the Rhizobia begins to show up; e.g. on the main root there is only a small extent of root nodule formation, while on the laterals developing later the number of root nodules is considerably higher. Such influence is exercised, besides Phygon, by Ceresan.

In the cortical cells of the rhizodermis of the main root the fungicides are supposed to accumulate to some extent and exert an inhibiting effect on the penetration, establishment and action of Rhizobia. It is all the more possible because the storing zone of the root is primarily the tissue zone of the cortex where the Rhizobium species establish themselves. This further explains the more intensive root nodule formation in the lateral roots. The amount of fungicides taken up per unit surface decreases with the branching off of the laterals, so in laterals developing later the inhibiting effect will be reduced. Another reason is that the laterals develop from the central cylinder inside the cortex, being thus in contact with the cortex only in a short section when they pierce it at the beginning of their development. So fungicides pissibly stored there in larger quantities get inside the developing laterals only in small amounts — if at all.

On this basis it would be worth carrying out chemical investigations to find out whether the organs — main root, laterals and aboveground parts — of the developing seedlings contain some — and if so how much — of the fungicide used in the treatment. Furthermore, in plant species where the radicle is sufficiently strong — e.g. of 1—1.5 mm diameter — the cortex and the central cylinder could be separated and the amount of fungicide determined in both tissue zones.

Even if no root nodule is formed on the main root under the influence of the fungicide, the possibility of Rhizobium species establishing themselves in several cells is not excluded, though they are not strong enough to develop root nodules. It would be desirable to perform microscope studies — e.g. below and around the root neck, where the root nodule formation is the strongest under control conditions — to find out whether some Rhizobia have established themselves or not in the cells.

Another question is the extent to which the fungicides effect the development of the plants in a positive or negative direction. It is a well known fact that in plants developing well and quickly the root nodule formation is much lower, as the type of metabolism in this case prevents the penetration of root bacteria; in plants of poor development nitrogen deficiency occurs which enchances the penetration of bacteria. Thus, besides a marked inhibiting effect shown by the fungicides, some of them may display a certain degree of growth stimulation too, which — on the basis of what have been said — may inhibit root nodule formation. The inhibiting effect fungicides have on root nodule formation may be decreased if phosphorous compounds are applied, since by a proper utilization of the phosphorus fertilizers the number of root nodules can be increased 4—6 times under control conditions. Thus, phosphorus application during the fungicide treatment can be expected to have a favourable influence on root nodule formation.

Just another suggestion. It would be desirable to make a morphological analysis of root nodule formation too in the case of various treatments. Namely, the different fungicide treatments, besides their external morphological effects, may change the tissue structure too, which could be analysed by investigations of this kind.

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CAN THE LOW YIELD OF SOYBEAN BE PARTLY CAUSED BY AN INSUFFICIENT RHIZOBIUM ESTABLISHMENT AND ROOT NODULE FORMATION?

Environmental protection is undoubtedly one of the most urgent problems of our times. The increasing number of scientific publications of this nature, the articles published almost daily in the newspapers, the delivered popular and scientific lectures all prove that reducing the pollution of the biosphere to a minimum is a question that engages public attention to a great extent. The most important field of environmental protection in agriculture is plant protection, more precisely: the application of pesticides. A decisive factor of the new plant protective research is to produce such non-persistent or specific chemicals which can be applied against pests or pathogens without doing any considerable harm to the natural environment. Extensive research work is carried on, further, with the aim of reducing the pollution of the environment by using the already existing pesticides more reasonably (e.g. by spraying on fewer occasions on the basis of appropriate forecasts).

Environmental protection research in Hungary is at a rather initial stage as yet, therefore publications of this character are mostly restricted to laying down general principles. The publication of M. Kecskés and J. M. Vincent is welcomed as an exception in this respect. Although the authors do not emphasize the importance of their research work from the point of view of environment protection, still it is perhaps the most significant of their work. In the experiment series carried out with reliable and easily reproducible methods *Vicia sativa* plants as well as appropriate *Rhizobium* strains and *Thanatephorus cucumeris*, a fungus causing root- and stem rot were used. The results unambiguously prove that of the seven commercial fungicides used for seed treatment only two can be applied without causing damage to *Rhizobium*, the useful microorganism studied. This finding deserves great attention since the yield of legumes may depend decisively on the successful establishment of N-fixing bacteria.

I sincerely hope that the authors will continue this work with the same thoroughness and after the *Vicia* extend their investigations to include other economically important leguminous plants too. I have in mind the soybean first of all. This plant seems to be a highly promising source of protein, at the same time its production is far from being solved in Hungary. The poor stands and low yields may easily be caused — at least partly — by the insufficient *Rhizobium* establishment and root nodule formation.

Finally, I should like to call the authors' attention to something not closely related to the subject, still affecting the value of the publication. The paper mentions a pathogenous fungus: *Thanatephorus cucumeris*, several times. I do not underesteem the knowledge of Hungarian experts by supposing that hardly any of them know this pathogenous fungus by this name. Even those who look it up in well-known international phytopathological abstracting journals will not get much help, since this name cannot be found in them. True, the genus *Thanaetphorus* Donk, and the type species of the genus: *Thanatephorus cucumeris* (Frank) Donk were already described in 1956, nevertheless this name has hardly become generally known. This frequent soil-borne fungus causing root- and stem rot is known by most experts even today by the name *Corticium solani* (Prill. et Delacr.) Bourd. et Galz., or rather as the imperfect stage of this basidiomycete: *Rhizoctonia solani* Kühn; this name is found in the scientific reviews as well. For proper information, therefore, the generally known synonym ought to have been given in the paper.

The pioneer publication of M. Kecskés and J. M. Vincent is an important contribution to the concrete experimental research work done in the field of environmental protection.

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WHAT IS THE EFFECT OF CHEMICALS IN MODIFYING THE MICROBIOCOENOSIS?

"With the increasing use of chemicals in agriculture non-biological control methods now play a part in the transformation of nature by substituting artificial agrobiocoenoses for natural associations" — writes Ubrizsy in a general study of pesticides in 1969. Though the effect of chemicals in increasing rentability is well known, our knowledge is very limited as to their share in modifying the microbiocoenosis. Thus, the reasonable utilization of chemicals depends on our better knowledge of their secondary influence on soil organisms. The nodulation on the roots of legumes presents an ideal model for studying ecologically important relations between plants and microorganisms. Though *Vicia sativa* has but a poor specification for races of *Rhizobium*, the remarkable results of Kecskés and Vincent have a basic importance in studies about the establishment of symbiosis in a micro-environment disturbed by the application of fungicides. This is a most important feature of the work even if it is not the purpose of the authors.

As to the experiment proper, with its exactness it fully meets the requirements of general microbiology. The elaboration and execution of the experimental methods are exemplary.

The studies begun in a labroatory and completed by tests approaching natural conditions as near as possible gave pratical results of great value. Due to a rapid method it becomes possible to choose fungicides compatible with inoculation. In this respect, however, a short review as to the active agent of the chemicals used would have come in very useful for the readers not familiar with the nomenclature commonly adopted in phytosanitary works.

A comprehensive review of the literature and the latest observations of great originality make the work complete which is indispensable for soil microbiologists and agronomists.

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WHICH PESTICIDES HAVE NEGATIVE EFFECTS AND UNDER WHAT CONDITIONS?

The increase of the production of vegetable protein by introducing new, economically justifiable methods has become a very important question in agriculture. Chemical means used for plant protection have taken such a position in the complex of agrotechnical operations that it is almost improbable they can be eliminated although their bad side-effects on soil biocenosis are well known. Therefore, there is a great demand for works explaining which pesticides may have a negative effect and under what conditions. It is very important, too, to determine the balance of the positive and negative action of a pesticide.

These questions underlie Dr. Kecskés and Vincent's work and justify its purposefulness. The authors compare the action of several fungicides on Rhizobium leguminosarum and symbiosis with papilonaceous plants. The action of fungicides on bacteria and symbiosis were observed and measured in laboratory experiments and under soil conditions. This system of the research was most purposeful, as it allowed the authors to obtain a closed whole.

They analysed, too, the usefulness of different methods for examining the activeness of several fungicides, as the latter appeared to be differentiated in this respect. When comparing the action of different fungicides it is desirable to use the same method, but when estimating the effectiveness of respective fungicides under different ecological conditions or regarding different organisms the correctness of results is conditioned by the selection of a proper method. From this point of view the authors' observations are valuable.

The right analysis of the results lets the authors arrange fungicides according to their agricultural usefulness. In this, Thiram has taken the first place as it best serves the function expected from this type of preparation.

In my opinion Dr. M. Kecskés and J. Vincent's paper "Compatibility of fungicide treatment and Rhizobium inoculation of vetch seed" deals with a live question in agriculture, and so it meets the requirements of publication. The authors have answered their own questions and obtained theoretically valuable results which can also be made use of in practice.

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CHRONICA

THE HISTORY OF THE CENTENNIAL BOTANICAL DEPARTMENT OF THE HUNGARIAN NATURAL HISTORY MUSEUM

The Hungarian National Museum has played an eminent role in the cultural life of the nation since the beginning of the nineteenth century.

The National Museum, a unique institution of this type at that time — in 1802 and 1808 — gradually gathered the material of special collections, later organized as wholly or partially distintc museums. Thus on the instigation among others of P. Kitaibel, botanist, the Collection of Naturalia and Artifacts, founded in 1810, and to be segregated in 1870 into three special units belonged to the Museum. One of these was the Botanical Department of the Hungarian National Museum.

The Botanical Department celebrated the centennary of its existence in 1970.

The three partial collections incorporating the natural history collections became an independent museum first during the Hungarian Soviet Republic in 1919, and actually in 1934, as the National Natural History Museum.

Between 1810—1895, one botanist each worked in the Botanical Department, namely I. Frivalszky, J. Sadler, Gy. Kováts, V. Janka, and Gy. Istvánffi; during the years 1896—1952, the number of research workers were usually seven; of these the more eminent ones (now deceased) were N. Filarszky, J. B. Kümmerle, S. Jávorka, G. Moesz, E. Gombocz, V. Kőfaragó-Gyelnik, G. Andreánszky.

In 1970, twelve research workers — museologists were active in the Department, and three pensioned colleagues are still working part-time: V. Csapody, E. Kol, L. Vajda. There are 9 technicians (preparators, laboratory staff), 2 librarians, and 2 caretakers. Thus the total number of the Departmental staff is 37.

The Botanical Department submits an annual report on its activity, and also prepares its annual work plan, to the Director General of the Hungarian Natural History Museum and Hungarian Academy of Sciences. The annual research project is a part of the 3, 5 and 15 year plans.

Within the nationwide scope and interest of the Natural History Museum, the Botanical Department preserves, in a conserved state, a representative partial set of the plant species of the world and more closely of Hungary. In this respect it fulfils a unique function in Hungary.

Its basic task is the augmentation and preservation of plant collections as bases of reference; to process according to present day requirements groups of plants by taxonomic and floristic means, and to investigate interrelationships between populations of species and their environment. The elaboration of the collections renders possible the utilization of results in public education.

Accordingly, the duties of the research worker — museologist can be summarized in three main groups: the enhancing and conservation (on the basis of predetermined principles) of the plant collections; the scientific elaboration of the collected material, and the furthering of public interest by the promulgation of results, mainly by exhibitions.

*

Research work. The first and longest chapter deals with the floristical research of flowering plants. The summary of results, Jávorka's book "Magyar Flóra" *(Flora Hungarica)*, of outstanding importance both as to scientific research and Hungarian cultural history, was published in 1924—1925. As its supplementation, the fascicles *Iconographia Florae Hungaricae* were published between 1929—1934. Among the sporiferous plants, the higher fungi were dealt with by Bohus—Kalmár—Ubrizsy ("Magyarország kalaposgombáinak meghatározó könyve") in 1951, with the senior author, G. Bohus, working in the Botanical Department. Concurrently with the floral works and identification books on plants, the taxonomic study of a great number of genera was also performed. Thus Filarszky processed the genus *Chara;* Moesz the home species of innumerable fungal genera; Kümmerle the vascular cryptogams genera; Kőfaragó-Gyelnik the families *Lichinaceae*, *Heppiaceae*, *Panniaceae*, *Peltigera*, *Parmelia*, genera; Verseghy the *Ochrolechian* genus of lichens; Bohus the genera of *Agaricales* and the genus *Hebeloma;* Ujhelyi the genera *Sesleria* and *Koeleria;* Radics the genus *Nymphaea;* Halász the thermobiont algae. Floristical phytogeography was a constant companion of floristical and taxonomical research work, leading to the study of plant coenoses and their inherent and regulating laws. In these studies, the forerunners were the German Warming, Grisebach, Gams and the French Braun-Blanquet. Working in this field, Zólyomi described the "dolomite phenomenon", the zonality of the vegetation in the Bükk Mts.; Fekete the insular occurrence of the cold continental forest-steppe wood in the hilly region of Gödöllő; Szujkó-Lacza pointed out the existence of the zonal montane — submontane beechwood in the northern part of the Central Range; Pócs verified the home occurrence of the Sarmatian mixed forest zone; Jakucs elaborated monographically the SE European karst shrub woods; Debreczy gave a detailed analysis of the oakwoods of Illyrian descent.

Qualitative coenological investigations demand the necessity of quantitative coenological and also quantitative ecological studies. On the basis of hitherto achieved results the Botanical Department also joined, in 1967, the work done in three sections of the International Biological Programme. In the Section PP of the IBP the photosynthetic activity of lignifying shoot axes was successfully demonstrated by functional anatomical, ecomorphological and physiological investigations in 1970 (Szujkó-Lacza—Fekete—Faludi-Dániel).

Public education. The first, independent exhibition of the Botanical Department was opened in 1953, under the title "Magyar növénytani kiállítás I.", with the final part in 1956. It displays the evolution of the flora and vegetation from the Tertiary to the present day in Hungary, further our present home plant associations, the anatomical structure and basic physiological functions of plants, the edible and poisonous fungi, fungal pests, and fungi serving for the extraction of basic pharmaceutical substances, — the whole exhibited in a lyriform hall of the Castle Vajdahunyad.

Apart from the permanent exhibition in Budapest, several travelling displays of the vegetation of phytogeographically uniform regions and their faunas were shown in larger towns between 1952—1970. By modern technical means these displays achieved extensive public attention. The same purpose was also served by the construction of some temporary displays within the frame of the permanent exhibition, usually treating a central theme. The travelling exhibitions are very popular owing to their easy transportability and theme of common interest — so far 5 have been made by the Botanical Department.

Among the popularizing handbooks, Jávorka — Csapody's "Flowers of Forest and Meadow" (published several times at home and abroad), and Bohus—Kalmár's "Fungi of Forest and Meadow" had the greatest success.

History of plant collections. The primary task of the research worker — museologist is the augmentation, and preservation of the plant collection, as well as its scientific and historical evaluation. After the presentation of the recent state of the Botanical Collection, a brief summary of the more significant collections will follow below.

General view. All collections of the Botanical Department of the Hungarian Natural History Museum are deposited on the first floor and partly on the ground floor (an area of 1.351 m^2) of Castle Vajdahunyad, Budapest; the library has two special rooms (193 m^2). The collections are preserved in 243 modern aluminium cabinets, in addition to 34 larch and some oaken cabinets, with the rest housed in cabinets originating from the Haynald legacy (Figs 1—4).

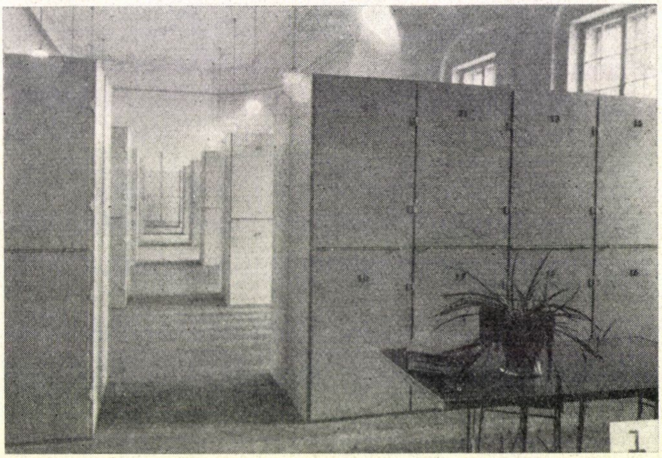

Fig. 1. Herbarium Carpato-Pannonicum (G. Szegvári)

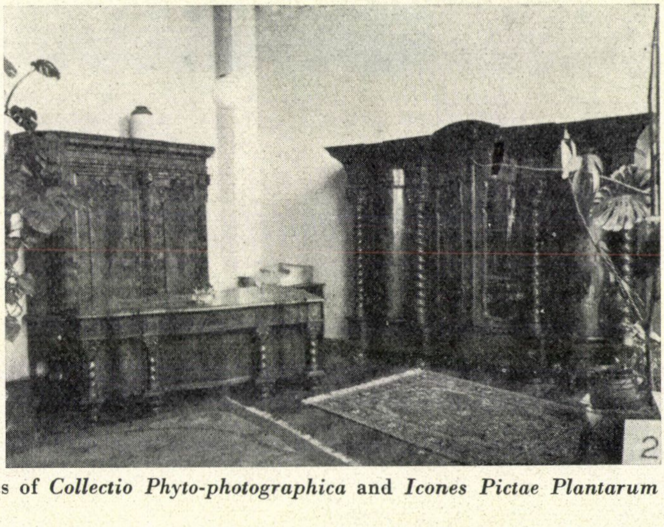

Fig. 2. Cabinets of *Collectio Phyto-photographica* and *Icones Pictae Plantarum* (G. Szegvári)

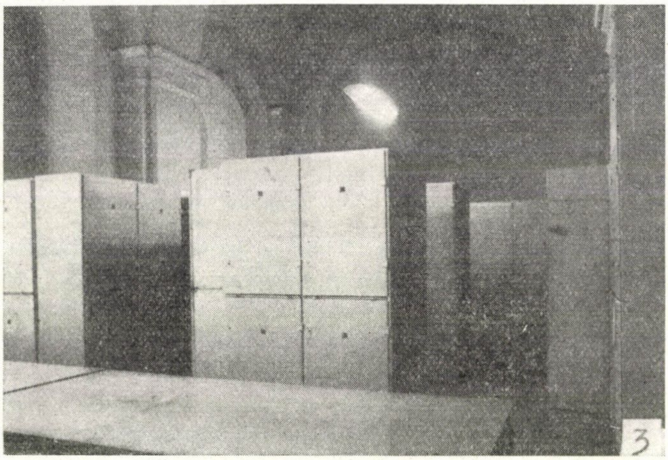

Fig. 3. Collectio Macro- et Micromycetum and *C. Lichenum* (G. Szegvári)

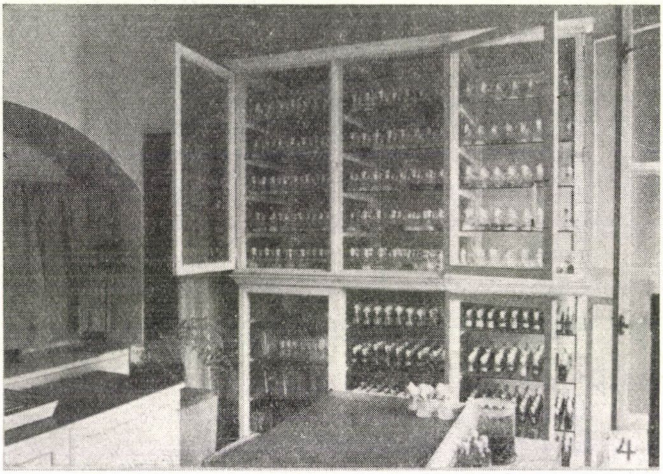

Fig. 4. Collection of living algae (G. Szegvári)

Inventoried (register books and cabinet cataster forms are obligatory) and estimated collection specimens totalled 1.313.416 sheets, capsules, etc. by the end of 1970. Within the collection cabinets the flowering plants are fixed (glued) on 32 cm wide and 44,5 cm long paper sheets, with the specimens belonging to the same species held together by a blue cover in all fascicles. The material of the non-flowering plants are preserved in capsules in accordance with the above principle, in green cartons; the collection of seeds is stored in plastic bags, the fruits in boxes. The dendrological material is placed in glass-door cabinets, the paleontological material (in drawers) in cardboard boxes per specimen.

As to the size of the material of the Botanical Department, it is probably among the ten biggest ones in Europe. In Central Europe, the collection is the third or fourth largest one.

In the Herbarium Carpato-Pannonicum, the vegetation of the Carpathian Basin is, on the basis of earlier collections, the most completely represented even today.

Until 1945, the Herbarium Generale was the richest in the world in Balkanian plant species. Now the Bulgarian and Yugoslavian collections are more complete with respect to these areas, than the respective material of the Herbarium Generale.

Special part. A brief summary of the history and specialities of the plant collections.

The first plant collection, consisting of some fascicles, is mentioned as early as 1810. After Kitaibel's death (1817), his collection was purchased and thus a valuable collection of the home flora became the property of the Museum. In 1820, the flowering plant collection rapidly expanded by the activity of the botanists J. Sadler and I. Frivalszky. In 1839, the Museum bought Sadler's collection, now enriched, owing to exchanges, by foreign herbarial materials. Sadler's large-scale augmentation of the collection derived from his intent of writing on the Hungarian flora of that time.

The collection material accrued in the Collection of Naturalia necessitated in 1870 besides the Zoological and the Mineralogical-Paleontological Departments, the creation of the independent Botanical Department.

During his earlier collecting trips to the Bánság, South Hungary, V. Janka accompanied L. Haynald. It was Haynald who created the first research officer's post in the Botanical Department, and V. Janka was appointed to fill it. Janka earlier also collected beyond the boundaries of the country, but the Haynald Foundation — 12.000 gold crowns — made collections possible in Greece, Italy, and Malta as well. By the description and deposition of new species in this collection, among others, of *Colchicum hungaricum* and some Balkan species, Janka called international attention to the Botanical Department. Tauscher's and Hazslinszky's collections were also obtained at that time (1883), enhancing the material of almost every collection, — which later became independent — and this holds especially for Hazslinszky's herbarium. Problems of storing the collections and the library now also arose.

Janka passed the care of the collections to Gy. Istvánffi. The new keeper worked first with algae and then fungi, and united the non-flowering plant collections into the first, independent collection of cryptogams. In 1892, the collection of the Department was greatly augmented by the Haynald legacy: "I am writing this Testament in my right mind and of free will . . . I do not own money or payments to anybody . . . beyond Kalocsa no one should look for any wealth of mine . . . I bequeath my herbarium and botanical and book collections, together with their cabinets, to the National Museum . . .
Budapest, 22 May, 1882 Lajos Haynald m.p. Archbishop of Kalocsa."

The inventory of the legacy shows a much later date (1898); according to this, there were 1721 books and manuscripts, 117.508 herbarial sheets, and 30 fascicles of unidentified material.

By purchases and exchanges in addition to his own collections, Haynald increased his herbarium to such a scale that it soon necessitated the geographic grouping and separation of the flowering plants.

Istvánffi's successor, N. Filarszky reported on the ordering of the herbarium and te grouping of the material (1899—1901) as follows: "The old main collection of the Department is placed in two rooms, Haynald's European collection also in two rooms, while Haynald's overseas collection in a separate room; Kitaibel's and Kossuth's herbaria were placed in a separate small room." (The Museum purchased Kossuth's collection in 1894; it has chiefly a historical interest, since there are no exact locality data. On the other hand, Kossuth entered profuse notes on most herbarial sheets, indeed, in several cases he was the first to record the vernacular (Hungarian) name of the plant.)

These collections contain not only flowering plants but in most cases also cryptogamous material. Therefore when the collections were re-ordered, the original date of increase of several — later independent — collections were found to be from earlier times.

N. Filarszky joined the Botanical Department in 1898. On the basis of his experiences in foreign countries and the earlier concept of Istvánffi, he proposed the establishing of a *Her-*

barium Hungaricum (at the time of its realization called *Flora Hungarica*, later *Flora Carpato-Pannonica)*, a *Herbarium Budapestinense* (not realized!), and the *Herbarium Generale* (later *Flora Generalis)*. The collections of Kitaibel, Mygind, Crantz, and Kossuth remained separate.

Filarszky's aim was the ordering and augmenting of the collections and the library. Moesz (1934) stated in his necrologue on Filarszky that "The enhancement of the collections and library is significant. By the thirtieth year of Filarszky's leadership the stock of the herbarium and the library doubled . . . in 1901 the total of plant fascicles was 2304, the number of books in the library 6750. When Filarszky retired, there were 5710 fascicles (that is, about 618.000 sheets), and 14.176 library items."

The collection Flora Hungarica (today *Herbarium Carpato-Pannonicum)* began to be established, according to his plans, in 1899, from the newly conserved and recently collected home plant species. The Hungarian material of the following large private collections — acquired by purchase, in exchange, or as presents — were incorporated in this collection; Barth, Csató, Degen, Kovács, Lengyel, Margittai, Simonkai, Szépligeti and Wagner. The *Flora Hungarica* now contains 334.072 sheets of flowering plants and 240 types.

The Flora Hungarica exsiccata, containing both flowering and cryptogamous species, increased by exchanging the species of the *Flora Generalis* and the collections of crpytogams.

The administration of collecting, ordering, exchanges and loans was mainly the duty of the keeper of the collection. The keepers of the *Flora Hungarica* were: S. Jávorka, B. Zólyomi, G. Andreánszky, P. Jakucs, and today G. Fekete.

The *Herbarium Carpato-Pannonicum* served as the basis for the writing of Jávorka's "Magyar Flora" (1924—1925), the handbook Soó—Jávorka: "Magyar növényvilág kézikönyve" (1951), and partly of Soó's "A magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve (1964—1973) *(Synopsis systematico-geobotanica Florae vegetacionisque Hungaria)*, from which the last volume is just being prepared. The Slovakian flora series, now under preparation, also took many of its floristical data from this collection.

The collection *Flora Generalis* (now *Herbarium Generale)* was re-created, by the unification of the flowering plants of the old main collection and Haynald's European and overseas materials, in all probability between 1910 and 1920. However flowering plant herbarial material from overseas already existed in the Department of Naturalia of the Hungarian National Museum in 1822 — the origins of this collection can be traced back to that time.

The earlier three distinct collections, the herbaria of home and overseas flowering plants and the material of the cryptogams, were ordered according to older systems, the new flowering plant collections to Engler's; the genera were provided with Dalla Torre numbers.

Nearly all of the larger collections, enumerated when discussing the *Flora Carpato-Pannonica*, contained plants from abroad; they enriched the material of the *Flora Generalis*. The following herbaria represented significantly large materials for this collection: Andrasovsky's and Degen's herbaria excelled in Balkan material, Haynald's and Kotschy's in African plants, while Sodiro's added extensively to the Ecuadorian collection. The herbaria of Staub and Dorner are also valuable additions in this respect.

After the earlier orderings of the collection as a common effort, among others Gombocz also incorporated, beginning in 1929, Weisz's collection from Asia Minor. Today the keeper of the *Flora Generalis* is J. Ujhelyi.

The herbarial material of the *Flora Generalis* increased, between 1900—1970, by 40.000 sheets of plants by purchase, 47.000 in exchange, 70.000 as presents, and by 5.000 recent collections. In 1970 the collection sheets totalled 503.020, placed in 81 aluminium cabinets in two rooms. The collection helped to complete the series *Flora Europaea* and the *Flora Bulgarica* with many data.

The several collections are interdependent, though the rate of interconnection and dependence is varying. Besides the *Herbarium Carpato-Pannonicum*, comprising the flowering plants of the Carpathian Basin as a geographical unit, and the *Herbarium Generale*, containing those (though naturally never completely) from nearly every part of the world, there exist several complementary collections of the various developmental phases of plants. Thus the *Collectio Seminum et Fructuum*, the *C. Dendrologica*, the *C. Plantarum Juvenilium Exsiccatarum*, the *C. Palynologica*, the *C. Plantarum Adventivarum et Cultarum*, the *C. Phytophotographica* and *Icones Pictae Plantarum*, preserving the results of technical or artistic perpetuation, or, again, the *C. Nomina Plantarum Popularium Hungaricarum*, the collection of Hungarian vernacular names useful in the preparation of systematic and ordering activities.

Passing over the mere mention of these smaller collections, the following discussion will briefly treat the several larger collections, on the basis of phylogeny and size, to wit: the *Collectio Pteridophytica*, *C. Bryophytica*, *C. Macro- et Micromycetorum*, *C. Lichenum*, *C. Algarum*, *C. Palaeophytica*. The history of the largest botanical basic library, in Hungary, that of the *Bibliotheca Botanica*, significant from the Departmental point of view, will also be given.

Collectio Pteridophytica. A significant part derives from Haynald's collection. In the unified collection of cryptogams, ordered by Istvánffi, the ferns already had their separate place. (J. B. Kümmerle was the research worker appointed by Filarszky as responsible for the *Equisetales*, *Lycopodiales*, and *Pteropsida*, in 1902. Kümmerle first elaborated Hollós's Caucasian *Pterydophyta*; for his revision, the Botanical Department received Weder's considerable collection of Vascular Cryptogams.) Kümmerle established the *Collectio Pteridophytica* in 1925. He made a separate file of all species present in the collection; this catalogue contained the respective floristical and literature data until Kümmerle's death in 1931. World War II brought no damages in the collection. When transferred to Castle Vajdahunyad, Jávorka ordered the collection, according to Christensen's system, in 1953. The recently accrued material was ordered by J. Szujkó-Lacza in 1957—1958, and the collection was placed in modern cabinets in 1969, ordered by F. Radics. The collection today consists of 30.000 sheets.

Collectio Bryophytica. The largest cryptogamous collection of the Botanical Department. Mosses were already present in Kitaibel's collection in 1817, but the first independent collection was established, with J. Szurák-Szepesfalvi as its keeper. The collection is among the significant ones of the world, because it contains the moss collection rich in Limpricht's types, acquired with Degen's herbarium, and the great majority of the documentary material of bryological collections and study results concerning the Carpathian Basin. The collection of mosses was ordered, according to Moenkenmeyer's *(Musci frondosi)* and Müller's (*Hepaticae*) systems, in 19 modern aluminium cabinets in 1970. The *Collectio Bryophytica* contains 105.278 capsules in the cabinet catastre. A special file-system aids the registration of the type material (holo- and cotypes). The keeper of the collection, L. Vajda, has recently retired.

Collectio Macro- et Micromycetum. The first fungus specimens came into the possession of the Museum in 1821. The collection attained its European fame under G. Moesz, its keeper, in 1906—1946. Moesz's main collecting areas were the environs of Lake Balaton, the Comitat Vas, the Sárrét, Budapest, the region of the rivers Ipoly and Garam, the High Tatra, Transylvania, the Velebit Range, the Croatian shores and Poland. The critical evaluation of his collections were published in the series *Fungi Hungariae*, while the work "A Kárpát-medence üszöggombái" was only published posthumously. After Moesz, the keeper of the *Collectio Micromycetum* was S. Tóth, followed by J. Gönczöl.

Hazslinszky's collection, purchased in 1889, represented a considerable part of the material of macroscopic fungi. The acquired material of the *Gasteromycetales*, elaborated by L. Hollós, is still probably the most valuable material of the *Collectio Macromycetum*. As a matter of fact, this collection, having no appropriate research worker until recent times, began its upward trend only after World War II. It is thanks to the Herpell-Bohus conservation technique that the fungus species can be stored, in perfectly preserved shape and colour, in a comparatively small place. In this respect, the collection is unique in the world. It was established by its keeper,

G. Bohus, and Mrs. M. Babos, technician. The stock of the *C. Macro- et Micromycetum* by the end of 1970 consisted of 48.124 inventoried capsules, and 4.000 unidentified or duplicate items. Both collections are in alphabetical order.

The *Collectio Lichenum* was founded by the purchase of the Hazslinszky collection at the end of the nineteenth century. The first lichenologist of the Department Gy. Timkó, established it as an independent collection. Its large-scale increase is the result of V. Kőfaragó-Gyelnik's work. By two independent exsiccata materials, Gyelnik also started international exchange, ably followed by K. Verseghy. Some larger lichen collections were purchased after 1945, thus the valuable materials collected by Szatala and Fóriss. The inventoried material of the *C. Lichenum* consists of 75.000 items, 3.000 duplicates, and about 30.000 unidentified capsules. The collection is the biggest in Central Europe; its type catalogue was published in 1964, followed by a supplementary edition in 1968. It is ordered according to Zahlbruckner's catalogue.

At an unknown date — according to the exsiccata materials perhaps in 1912 — Filarszky established a special collection of the genus *Chara* and maritime algae. In 1917, the collection was enriched by Pantocsek's *Diatoma* material of world fame. Filarszky wrote his *Chara* monography, still unpublished, on the basis of the material now known as the *Collectio Algarum*. After World War II, a preliminary ordering of the collection was made by M. Halász; its present day suitable arrangement is the work of E. Kol and Zs. Páricsay-Komáromi (1968).

The collection of algae now contain the following smaller collections: a herbarium (exsiccates), a conserved collection, microphotographs, microscopic preparations, and a collection of living algae. This latter was created by E. Kol in 1949, from her earlier collections. It contains both home and foreign material — among its curiosities are the algal species brought into culture from the green snow of the Antarctica. Its value as a study object is considerable. The most valuable part of the microscopic preparations is J. Pantocsek's several hundred slides of diatoms, rich in types.

Collectio Palaeophytica. From the first localities of the Hungarian Tertiary flora (Tállya and Erdőbénye), the fossil material was acquired by the Hungarian National Museum. Its discoverer and collector was Gy. Kováts. For ninety years thereafter there was no paleontologist in the Department. Beginning, however, with 1940, K. Rásky, and from 1953, G. Andreánszky converted it, with the help of their colleagues G. Szilágyi-Cziffery and E. Horváth, into one of the biggest European collections. Its material contains the documentation of the following monographs: "Die Flora der Sarmatischen Stufe in Ungarn", "On the Upper Oligocene Flora of Hungary; Analysis of the Site at the Wind Brickyard", "Die Oligozäne Flora des Kisceller Tons in der Umgebung von Budapest". The inventoried material of the fossil plant collection totals 18.142, the still uninventoried items run to more than 20.000 pieces. The number of described types is 202. The material is arranged according to geological periods, within these to localities, and in Hutchinson's system.

Bibliotheca Botanica. The first few books originated from the legacy of F. Széchenyi, the founder of the National Museum. In 1890, 758 books were inventoried. The library of the Haynald legacy consisted of about 6.000 volumes and papers, estimated at a value of 5795.65 Forints. The most valuable items were, e.g., Waldstein-Kitaibel's and Jacquin's great floral works, Kerner's *Hortus sempervirens*, Sibthorp's *Flora Graeca*, etc. Haynald's careful and regular subscription to periodicals was further made possible by his Foundation (12.000 Forints) for the Botanical Department. Until World War I, the books and periodicals acquired by the interest accruing from this Foundation made the library one of the foremost libraries in Europe. Between the two world wars, the regular increase of its stock was rather intermittent owing to financial difficulties. During World War II, a considerable part of the books, displaced for a securepreservation, were ruined. Besides purchases, the inner source of incrementation of the library since 1877 has rested in the Museum publication "Természetrajzi Füzetek," later called (as today) "*Annales Historico-naturales Musei Nationalis Hungarici.*"

Recently we published "*Fragmenta Botanica*," or under its present title, "*Studia Botanica Hungarica*," containing only botanical material. The value of periodicals received in exchange is considerable today.

Not only the librarians but also the Directors, and principally S. Jávorka, of the Botanical Department were zealous supporters of the enhancement of the Bibliotheca Botanica. Among the gifts received, I. Györffy's and B. Györffy's material of books and periodicals excelled by the value of their botanical content, greatly complementing our bryological literature and those on the newest branches of modern genetics in the *Bibliotheca Botanica*. J. B. Kümmerle was the first librarian; E. Till-Forbát is responsible for it today. The *Bibliotheca Botanica* now contains about 80.000 volumes and 240 current periodicals. Since 1946—1947, the *Bibliotheca Botanica* has been a part of the Central Bibliotheca of the Natural History Museum.

Acknowledgement

The author expesses his sincere thanks to the keepers of the special herbariums and collections, namely: G. Bohus, V. Csapody, Zs. Debreczy, G. Fekete, J. Gönczöl, D. Kováts, E. Kol, F. Radics, G. Szilágyi-Cziffery, E. Till-Forbáth, J. Ujhelyi, K. Verseghy and his assistant Gy. Mátéffy, K. Speckner.

J. SZUJKÓ-LACZA

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RECENSIONES

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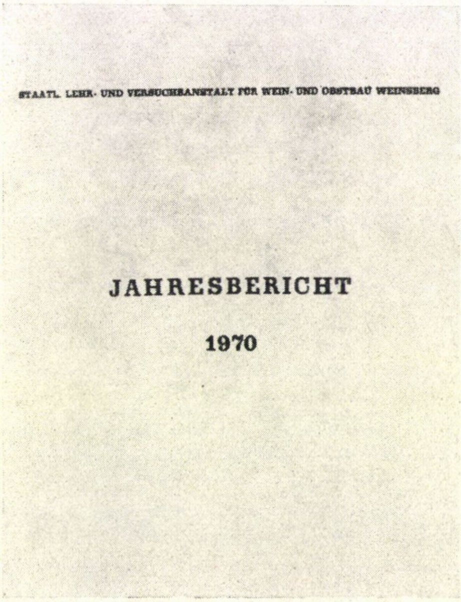


STAATL. LEHR- UND VERSUCHSANSTALT FÜR WEIN- UND OBSTBAU WEINSBERG

JAHRESBERICHT

1970

The Year-books consist of 12 chapters per each.

I. Staff and land structure.

Enumeration of the director and the personnel in the various offices and sections. Distribution by cultivation branches and area of the land of the Institute is included in the same chapter.

II. School and students' hostel.

Section leader: Orir W. jr.

1. Two years training of technicians. The course started for the ninety ninth time on 7th January 1969 with twenty students. A list of the students with the personal data is presented subsequently. A) Theoretical instruction. Enumeration of theoretical and practical subjects in each grade. B) Practical training. Number of hours and days spent in practical training in each grade. C) Examinations. The students sit for verbal and written examinations in the theoretical and practical subjects and having passed them obtain a certificate. With the breaking up ceremony the director takes leave of the students. One of the students delivers a lecture. It is on this occasion that students are given various prizes and decorations.

2. Cooper's training. Leader: dr. Schulle.

The training lasted in 1969 from 28 April to 5 July with the participation of 14 students. In 1970 the training course did not take place. This section also included a course in wine cellar management, where students were trained in 10 theoretical and practical subjects, and at the end of the year sat for a final examination in four subjects.

3. Short courses.

4. Competition for the best results.

III. Section of wine chemistry. Section leader: dr. Schmid. a) Tests performed. b) Yeast purification. c) Trainees. d) Scientific work. Owing to the very large number of the verbal and written tasks of the lessons and practices and of the samples sent in for examination there was no opportunity to carry on scientific work in 1969 and 1970.

IV. Vine breeding. Section leader: dr. Schleip.

1969:

a) Variety maintenance. The aim was to select suitable clones from varieties grown in Württemberg and prepare them for propagation. Selection made earlier had not been satisfactory due to the high degree of subjectivity domineering during the procedure. The destruction of the individuals selected was the result of insufficient care. The area available earlier in Württemberg proved to be too small for really efficient work. Experience showed that three times as large an area as the earlier one was necessary. Canes have to be evaluated several times a year which requires labour performed by a number of workers. The main emphasis is laid upon the varieties Trollinger and Rizling.

Proper selection of clones took place only with the variety Burgundi — due to the extremely high variation capacity of this variety. In the case of other varieties selection was made at random.

b) Breeding by crossing. The aim was to produce a wine grape containing the taste matters of the best southern varieties coupled with a sufficiently slow ripening which enables the deevlopment of a suitable acid content.

The number of crossings was reduced on the basis of wine qualification made during 1962—64, and in 1969 crossing was only performed between weisser Sauvignon and Rizling and weisser Sauvignon and Trollinger. Because of the low number of plants pollen was obtained from Verona.

In order to obtain a sufficient amount of "cover wine" (wine containing much colour substance) further crossing was performed. All the earlier varieties belonged to the variety Burgundi and their colour was much poorer than that of the Spanish wines.

In 1968 the only „rubintraube" variety available was crossed with the variety "limberger." In 1969 106 plants were used and fifty percent of them was rubintraube.

Seedlings were selected from a breeding plot. Some 700 plants were evaluated and propagated. On the basis of preliminary evaluations for the experimental area the variety "Helfensteiner" was crossed with the variety "herold," and a medium quality red table wine obtained. Growth, berry shape and ripening received a good qualification. Another red wine variety was produced from limberger x schwarzrizling, and a white wine variety from rulander x rizling. The variety "Marzemino" was not accepted.

1970:

a) Variety maintenance. The earlier applied methods of clone selection proved unsuitable. The more than one year old germs were infected by viruses. In order to produce a sufficient amount of canes in 1972 four work groups consisting of young workers were created. Selection was carried out with great care. Virus diseases were shown most intensely by the vascular tissues of the leaves; plants where the symptoms of the disease appeared on a 1—2 mm section of the vascular leaf tissue were excluded from further breeding. In serological studies performed the following results were obtained.

1. It is mainly in the period of leaf development that the virus causes considerable physiolopical damages. The symptoms appeared mostly at the end of June and beginning of July first of all on leaves above the clusters, while in autumn on axillary shoots too. In many cases the symptoms disappeared in autumn although the plant was infected.

2. In the critical period symptoms were observed in all varieties.

3. A single cane of a variety which was infected by some virus exposed not only the other canes but also the other vine varieties to danger.

4. By thorough selection the virus diseases can be successfully eliminated. However, selection should be more thoughtful in the future.

5. The selection of virus-free plants has not been solved so far. In a snumber of varieties selection is restricted to a single vine-stock.

b) Breeding by crossing. Crossing by hand pollination in heated polyvinyl tents was highly efficient with the variety limberger. In 1970 high yields were obtained in the trial plot.

On the basis of preliminary test results four red and two white wine-grape varieties were given state certifications. In addition, two blue- and two wine varieties were qualified as of medium quality.

V. Vine and vine-graft

Section leader: Kümmerer

1. Weinsberg, 2. Talheim, 3. Wildeck, 4. Gundelsheim, 5. Lauffen, 6. Offenau. The main characteristics of the vegetation period, phenological data as well as those of plant protection, irrigation and yields for all six growing sites are presented subsequently.

VI. Fruit growing

Fruit yields and phenological data per fruit species and treatment in Winsberg and Heuchlingen are presented.

VII. Wine cellar management

Must- and wine storage in Winsberg, Wildeck, Gungelsheim, Offenau and Lauffen are described, and wine prices given.

VIII. Advisory service. The 23rd advisory session was held in 1969, and the 24th in 1970.

IX. Lectures. In 1969 31 while in 1970 41 lectures were delivered.

X. Publications. In 1969 15, in 1970 7 publications were issued.

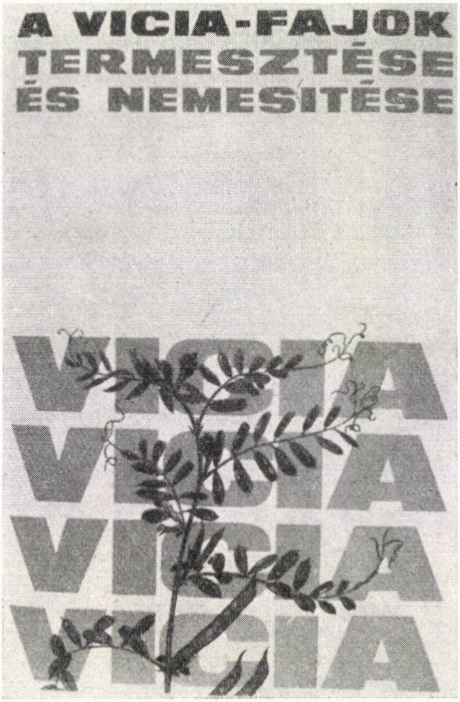
XI. Climatic data. Temperature, precipitation, number of sunshine hours, number of frosty and icy days are included.

XII. Experiments and researches of 1. Wine cellar management, 2. Fruit growing and 3. Vine growing are described.

Gy. Borka

A Vicia-fajok termesztése és nemesítése (Growing and breeding of Vicia species). (Ed.: A. Jánossy), Akadémiai Kiadó, Budapest

In the past months a highly valuable and interesting volume was added to the Monography Serie of the Department of Agricultural Sciences of the Hungarian Academy of Sciences. Namely, a work was published written by A. Jánossy: "Growing and breeding of *Vicia* species (vetch production)." This book is considered to meet a long felt need, as it sums up the knowledge of a plant genus not often dealt with in the literature on the basis of the most recent research results and practical experiences.



The genus *Vicia* covers some 150 species the overwhelming majority of which are wild species and only a few of which have entered the lines of cultivated plants. The cultivated plants among them are: the horse bean, the common vetch, the hairy vetch with two varieties, and the Pannonian vetch. Their economic value — and consequently their utilization, too — is different. Their production is not general, it mostly takes place in isolated centres. There are species which can be considered as ancient cultivated plants, while others have only recently been introduced to production. Thus, e.g. the horse bean and the common vetch are old cultivated plants, while the hairy vetch and the Pannonian vetch have become field crops in the most recent times.

There are species within the genus *Vicia* which have often drawn attention to their usefulness and attempts have been made to produce them, or else, even of they were established as field crops were only the subjects of repeated production attempts and have been introduced, only, in small districts. Thus e.g. the book mentions the *Vicia narbonensis*, which is produced, here and there, all over the world, and according to Sándor Cserháti was produced for years at the end of the 19th century in the domain of Lepsény.

Attempts have been made to grow *Vicia dumetorum*, *V. cracca*, *V. sepium*, *V. striata*, etc. too. However, they have not met the expectations. The production of *V. monantha* and *V. ervilia* has been maintained to some extent under extreme conditions, mainly in Eastern Europe. The production of *V. angustifolia* in the Mediterranean regions and Western Asia, of *V. articulata* in West- and South-West Europe, of *V. benghalensis* in North-America can be met sporadically.

There are outstanding morphological differences between the Vicia species and, consequently, their production requirements and methods are also different. Thus e.g. the differences are great between the horse bean and the common vetch or other vetches. The differences are similarly great in their production purposes and area of utilization. E.g. the horse bean is definitely a grain fodder, used for human consumption to a small extent, while the other vetches are used as green fodders, silages, or to a small extent — as in the case of common vetch — as hay.

The interest shown in their production also manifests itself in different degrees. Thus, due to its high climatic requirements the horse bean is the plant of more humid sea coasts and its production in Hungary is very limited. New interest has been raised towards it, however, with the protein problem and the necessity of its solution coming into the limelight. With the extension of irrigation farming its role may increase.

The production of common vetch was wide-spread, moreover, before the introduction of perennial Papilionaceae in the past period it was the most important rough fodder grown in the field. However, due to its single crop it is expensive, although, as a result of its early harvest preceding the period of drought, it gives relatively reliable — though not large — yields. The production of mixed winter fodders of hairy- and Pannonian vetches is gradually increasing as a result of the wide possibility of their application, since — due to their early harvest — they almost certainly make a second crop possible, and so provide a large amount of nutrient per unit area. Under Hungarian conditions a further increase in the production of hairy- and Pannonian vetches is a serious and well-founded objective.

It should be mentioned that — except the horse bean — a considerable seed production of the cultivated vetches is carried on in Hungary, not only to meet the inland requirements but also for export purposes. The interest of farms is indicated by the fact that in the framework of contractual seed production the planned area of common vetch was exceeded by 160 percent, that of the hairy vetch by 201 percent and the area of Pannonian vetch by 155 percent, even in 1966. For this very reason the seed cleaning of vetches is also organized, and there is a raw material processing capacity of about 10.000 tons available. The separation of the seeds from those of the supporting plant is generally carried out at the site of production, whereas up-tp-date base machines, high capacity horizontal disc separators, rubber machines and separators are available for the cleaning of vetches. Several hundred wagons of vetch seed are exported annually, the overwhelming majority of which — even today — is common vetch. In this field France and Bulgaria are the rivals of Hungary. The great interest in vetch seed is shown by the fact that, apart from the socialist relations, there are Hungarian exports to the German Federal Republic, Austria, Italy, Holland, Denmark, the United States of America, and — though in smaller quantities — even to Japan.

The authors of the monography were well directed so the individual parts join each other logically and the adequate proportions are ensured everywhere. The style of the book is also perfectly uniform, and the individual parts are co-ordinated.

The work is divided into introduction and four main parts. Its special value is that it does not concentrate on the cultivated *Vicia* species, but deals with the wild species too, first of all with those occurring in Central Europe and Hungary.

The introduction discusses the agricultural importance of the *Vicia* species and their production, geographic position, the role and distribution of cultivated *Vicia* species.

At the end of each chapter a full list of references presents the related literature. This gives information about research results up to the present day. Its outstanding merit is that the exposé of literature, often unavoidable with monographic treatment, is pushed into the background in this case, because both the text and the tables and photos are based dominantly on the authors' own investigations. They include even such minor details as the value of components in the wild *Vicia* species, etc., which has little practical value today and serves only to ensure completeness.

The first part discloses the biology of the Vicia species with a preciseness so far unknown in the literature. In this part the section dealing with the taxonomy of the *Vicia* species, as well as the taxonomic key of the Hungarian species which includes the morphological features of seeds and seedlings are especially valuable. The chromosome relations of the *Vicia* species are discussed in a separate subchapter, since e.g. the horse bean — due to its low number of large size chromosomes — is highly suitable for investigations at the level of cells. It is a widely used test plant as to nucleic acid metabolism as well. Further on, the parts related to the external and internal morphology of the *Vicia* species, which even include the anomalies are outstanding. The authors' own research materials concerning the germination physiology, metabolic processes, evolution biology, flowering biology and genetic conditions of the Vicia species are excellently treated.

Part II discusses the relationship between the Vicia species and the environment. In this framework the climatic and edafic factors are dealt with comprehensively, in spite of the different requirements. The axis of the chapter is made up by the diseases and pests of the *Vicia* species and their control. This is quite new, since the Vicia species — being a rather neglected group of cultivated plants — have not been treated independently so far. This part is followed by the chemical weed control of *Vicia* species which — except for the horse bean — is not very interesting. Even in the case of the horse bean only foregoing attempts can be spoken of. All these parts are excellently completed — besides the photos, figures and drawings — by the coloured tables of K. Komjáthy, I. and Dr. Papp, E., which show a wide range of vetches, including the pathogens and pests too.

Part III is the axis of the monography discussing the growing of *Vicia* species, for feed mixtures and seed production separately. This part meant a difficult problem, the more so because the agrotechnical requirements of plants of totally different demands and type had to be co-ordinated. The part discussing the questions of production is followed by an evaluation of the tests and data on the value of the components in the *Vicia* species. This part includes the data of a whole series of wild vetches.

The last part, Part IV deals with the breeding methods and -results of the *Vicia* species at a very up-to-date level. This is all the more important, because the Hungarian research workers were pioneers in breeding the common- and Pannonian vetches and introducing them in production. The chapter on breeding is completed by a list of true-bred varieties, which is very comprehensive and also includes discovered local varieties. A good basis for this was the rich foreign collection and the material of the Hungarian local variety research at the Institute of Agrobotany. It might be mentioned that — probably due to the time at the printer's — the "Lippói" horse bean, the only certified Hungarian horse bean variety developed from a local variety is not mentioned in the book.

In our opinion, as an obstacle to the wide introduction of horse bean production, its high seed requirement per unit area and a consequent low propagation ratio should have been mentioned.

When speaking of vetch mixtures, besides the mixture of peas and vetches its joint production with lupine, too, could have been mentioned, which is used in North-East Hungary, and is rather wide-spread in Poland.

The book consequently uses the term "fodder vetch" which, in fact, may refer to any of the species. Perhaps it would have been better to stick to the name "common vetch" (or "spring vetch" as called in Hungary). For the sake of completeness it might have been mentioned that the common (or spring) vetch also has an autumn variation produced earlier in Hungary, too. Namely, according to earlier experiences, its winter-hardiness is equal to, or slightly better than that of the autumn peas.

All these, however, do not affect the internal value of the work; perhaps, it is only the rich presentation that increases the demands.

The monography is a great asset to agricultural literature, because it has disclosed an uncleared part of production giving guidance for a considerable time to theoretical knowledge and practical experience concerning the Vicia species.

D. Penyigey

P. Stefanovits: *Brown forest soil of Hungary.* Akadémiai Kiadó, Budapest, 1971.

Research on the alkali soils was for a long time the internationally most recognized branch of Hungarian soil science. Although we had noteworthy results in other fields of soil science, too, it was mostly in the last two decades only that the international scientific life began to show similar appreciation for them as for the alkali soil research. Pál Stefanovits played an important role in effecting this welcome change. He joined the work of soil mapping in Hungary in 1943, and in ten years completed soil maps of 350.000 ha area. Investigations made in the course of this work as well as the collecting and processing of the different data made it possible for him to become the leader of an up-to-date soil surveying work extended to the aspect of land utilization, too, on one hand; and — in the possession of a material required for the soil mapping — to elaborate with his collaborators a Hungarian soil system built on a genetic foundation, on the other.

Stefanovits's special line has always been the research of forest soils. With a thorough analytical work he cleared up the conditions for the development of soil types, the processes taking place in them and made proposals on their practical utilization, too. The importance of this work is shown first of all by the fact that a round 60 percent of the area of Hungary is covered by forest soils, and of them 36 percent is the share of brown forest soils. Of the 60 percent forest soils forests do not occupy more than 14—15 percent. From the above numerical data it is clear that a more detailed knowledge of the forest soils is important not only for the forest management; still greater advantages may result from it for the agriculture. Yields are highly varied on the brown forest soils, too; at some places 5—10 q/ha while at other places even 25—35 q/ha grain yield may be harvested. Thus, the classification of forest soils and the disclosure of their regularities are of great importance from an economic point of view, too.

This is the reason why Stefanovits's book is so valuable, though its role in making the results of Hungarian research known abroad is not less important either. Our language conditions mean numerous disadvantages for us, so any work that sums up briefly and concisely the results attained by us in some special field in a world language fulfils a great mission. It not only gives information on the present level of research work, but also calls attention to earlier literary publications which could not become generally known for the very reason of lingual difficulties. Therefore, Stefanovits's work has a great mission to fulfil from the point of view of science history, too.

The book is divided into four parts. In the first part the author gives a short review of how the system of soils — and of brown forest soils within it — has developed. He agrees with Tyurin's proposal on separating the brown forest soils into Western-, Central- and Eastern European facies; the brown forest soils of Hungary can be placed among the Central European and South-Eastern European brown forest soils. The author's main ambition is to prove the correctness of this classification in his book. The first part contains, further, a short description of the methods employed during the survey and the way of evaluation of data obtained.

The second part analyses the processes taking place in the brown forest soils and playing a role in soil formation. It deals with humus formation, leaching and accumulation, clay formation and — decomposition, clay migration, formation of "kovárvány",* processes of reduction and oxidation, acidification, processes of brown forest soils turning into steppes, soil and wind erosion and separately with the erosion of brown forest soils.

The Hungarian results are presented in comparison with the results of foreign, first of all, Russian-Soviet and German researchers. In this part a large number of results obtained by the study of the DTA curve of clay are given, too, and many good photos illustrate the regularities gathered from the microsections. The author was the first in employing widely these techniques in Hungary. Thus his exposition is very instructive not only concerning its content but methodologically, too.

A lot of genuine research results are contained in the chapter written on the formation of "kovárvány". László's attention was called to this phenomenon as early as in 1913, its detailed study was, however, performed by Stefanovits. He distinguishes four kinds of "kovárvány", of them the typical "kovárvány" develops in homogenous acidic sands, and in its formation the processes playing a decisive role in the development of brown forest soils, too, dominate. Development of predestined "kovárvány" layers is conditioned by an anisotropic parent rock and different geological stratification. Secondary "kovárvány" is brought about as a result of a very intensive clay infiltration. Its precondition is: a parent rock containing clay, in the more sandy layers of which, developing after the clay migration thin dark veins appear in the course of forest soil formation. Finally, the so called compound "kovárvány" develops when a coarse parent rock is mixed with a loess or other similar textured layer.

The author's investigations into the changes occurring in the brown forest soils under the influence of agricultural cultivation are very interesting. He studied soils

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* sandy soils marbled with ferric oxides

proved by old maps to have been under agricultural cultivation for almost two hundred years and compared them to one another and to the investigation results of similar nearby soils proved again to have been covered with forest for a long time. The influence of crop production is summarized as follows: The water supply is different due to differences in location between the roots of plants in the two cultivation branches. Under the influence of agricultural crop production the upper soil layers are drier but in the deeper layers moisture content is higher than in the forest soils. Organic matter contents show less than 0.5 percent differences in favour of the forest soils. Saturation under forests shows a downward increase from 42 per cent to 100 per cent; under arables higher saturation values can be found. Soil dynamics change, acidity decreases as a response to agricultural crop production. It would have been useful to complete the figures illustrating the periodical water contents (Figs 11—14) with the distribution of precipitation and the water regime characteristics of soil layers (water capacity, hy, clay content). These data are difficult to identify from the tables; in many cases data required for this are not even available.

The chapter which deals with the question of erosion discusses the effects of wind and water erosion and separately the erosion of brown forest soils. Information on damage done and on the erosion processes occurring is given by a map.

In the third part the brown forest soil types are described. After a brief morphological description the author presents the differentiating characters highly important from the point of view of identifying the individual types; special emphasis is laid on the proportion of clay content in the different layers. Then reference is made to the water regime of the types, and the characteristics by which the sub-units within the types — sub-types, variations — can be distinguished, are listed.

The clear definitions solve the problems of many organizers and supervisors of farms. It must be realized that in field work the knowledge of characteristic features is indispensable for the classification of transitional types.

Distinction made between variations on the basis of the thickness of the surface soil, which is very practical, and is fully supported by the practice of forest management. According to our experiences this factor decisively influences — besides the choice of the stand — the volume of wood yield, too. As for the future, attention is worth being paid in the case of certain brown forest soils (e.g. acidic, non-podzolic brown forest soils) to the percentage amount of detritic parent rock. This, too, often affects the volume of wood yield.

In the fourth part the areal distribution of brown forest soils is discussed. The author describes the system of regions and large-regions and characterizes them in a few sentences even when the proportion of brown forest soils within them is low. By doing so he gives the reader opportunity to get a comprehensive picture of the soil conditions in the country, and fit those told about the forest soils in this picture.

The book is completed with appendices referring to the text. Tables show the data of a detailed analysis of the characteristic soil profiles studied, from the description of the site to the laboratory analysis. Latter contains mostly the following: mechanical structure, specific weight, volume weight, porosity; other physical characteristics: pH, y_1, y_2, $CaCO_3$, hy, Arany's point of stickiness, humus, exchangeable cations, complete analysis of clay fraction, exhangeable iron, aluminium and silicic acid. Data not only produce proof to what is said in the text, but at the same time offer a basis for comparison and for classification into types, too. A short explanation of some analytical results would have served for clearing the uncertainties.

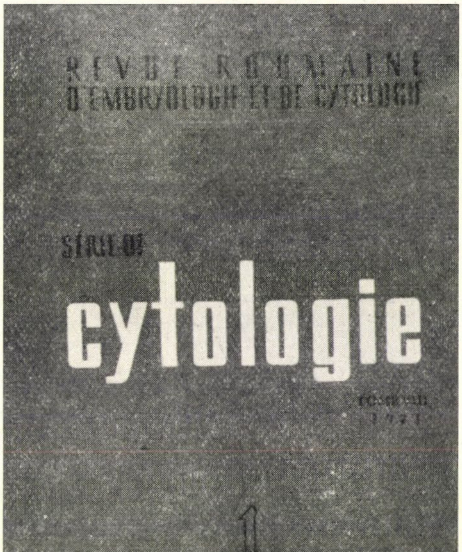
12 coloured photos show the characteristics of the soil types described. It is probably for reasons of printing technics that the coloured pictures form the only less successful part of the book. The overdomina-

tion of green and blue colours has sometimes a disturbing effect.

To sum it up, we can say that Pál Stefanovits's work is a brief and concise summary of our knowledge acquired so far of the Hungarian brown forest soils. It is a short but meaningful treatment of the subject. It gives the reader a full picture of the subject discussed, a survey of the most important foreign and Hungarian publications, and information on the abundant results of the decades of author's activity. It is an additional pleasure to its scientific value and practical utility that this highly important work has been published in excellent English translation.

Z. Járó, I. Szodfridt

Revue Roumaine d'Embryologie et de Cytologie Série de Cytologie. 1971. 8. 1—2.



The periodical is a publication of the Roumanian Academy of Sciences. Its aim is to publish original papers written on subjects of anatomy, histology, ultrastructural research and histochemistry. The papers are written in French, English and German.

Even a cursory review suggests that the publication is well constructed and has high requirements both for content and form. A thorough examination confirms this opinion. The introductory study of the first number of the volume is a work by the academician Marza and his group. The authors have been studying for some time the self-renewal processes of cells. In the present paper they discuss the correlations between the DNA content and size of nuclei in the course of granulocytopoiesis. The essence of the problem is, in fact, the interrelation between division and differentiation, which has always been a fundamental question of cytology. The granulocyte maturation series is a good model system for studying this problem. In the course of investigations the DNA content of nuclei and its changes in the members of the promyelocyte — polymorphonuclear cell series were determined with a cytophotometric method. On the basis of their data the mesoplastic and teleplastic phases of differentiation can be well characterized. In the former phase cells are still able to divide, while the latter is the phase of maturation without any division of cells. A necessary precondition of mesoplastic division is the reproduction of the DNA content (and not the doubled volume of the nucleus). The increase in the volume of the nucleus is not proportional to that of the DNA content. It is interesting that the correlation between DNA: nucleus surface is less pronounced in the mesoplastic period than in the teleplastic differentiation. It thus seems that the dimensions of the nucleus are determined by a number of factors namely — besides the DNA content — by a heterosynthetic activity, and that in a way characteristic of each phase of maturation.

The first number includes another paper written on cytophotometric methodology, a study of Aratei et al. on the DNA content of human oocytes. The authors point out that the oocytes may possess more than twice the DNA content in the lymphocytes (diploid cell). On this basis they discuss whether the priciple of the constancy of the DNA content can be applied to the given object.

It is a well known fact that the functioning of the individual organs and of the whole organism shows rhythmical (daily, monthly, seasonal) changes. This rhythm is also manifested in the activity and morphology of cells. In the first number two papers deal with this subject. C. Barbarasa describes the changes in the number and localization of nucleoli in liver cells as a function of feeding time. According to his observations the number of nucleoli is the lowest 6 hours after feeding, and reaches a maximum after 12 hours. The author brings the changes into connection with metabolic processes following the uptake of food.

M. Balan and L. Marza demonstrated rhythmical changes dependent on the type of nervous system and time of feeding in the division of epithelial cells of the gastric mucosa and in the eosinophil cell content of the mucous membrane. It is an interesting observation of theirs that the amplitude of daily fluctuations in the mitotic index depends on the type of nervous system: in highly mobile (sanguinic and choleric) groups it is wider than in the weak group. There is an inverse correlation between the functional activity of the stomach and the mitotic activity of cells.

In this number several papers deal with the morphophysiology of pathological processes caused by experimental injuries. The processes of calcium accumulation in the lesional centres were studied by L. Wasserman and his collaborators under the conditions of cardiovascular- and kidney lesions caused by dihydrotahysterin and sodium phosphate. According to observations made with autoradiographic methods only a low quantity of labelled calcium appears in the centres suggesting that the accumulated calcium salts originate from the reserves of the organism. S. Dolinescu et al. discuss the pathogenesis of atherosclerosis, D. Onicescu and I. Cuida the problems of anaemia and experimental siderosis in their respective papers.

There is a paper on endocrine in the first number too: a study by M.I. Hurduc and his collaborators. The authors described the qualitative and quantitative changes in the cells of the adenohypophysis after the extirpation of the thyroid gland.

The diversity of subjects is characterized — besides the above — by the fact that in this number a study is included on an invertebrate (T. Gheorghiu's paper on the muscle tissues of Bombyx mori larvae) and one by S. Ch. Roy on the karyology of various Scilla species.

The second number of the volume contains 15 papers. We cannot give detailed information on them here only wish to call attention to the subjects. The two introductory papers deal with the problem of liver cirrhosis. M. Banerjee describes the karyotype of three species in the family Lemnaceae. The study of I. Kiricuta and his collaborators who pointed out that some surface active substances induced metastasis is very interesting. M. Dragan et al. describe a rapid and simple staining method elaborated by them for the identification of cancer on the neck of the uterus.

Investigations made by Stoica and Ionesco who analysed the changes of 14 different — mainly lysosomal — enzymes in alveolar macrophages in the course of immune reactions and found the activity of lysosomal enzymes to be increasing — are worth being paid attention.

M. Gocan's paper is also an enzyme-histochemical work; he studied the activities of thyroid succindehydrogenase, ATP-ase and alkaline phosphatase after TSH treatments.

In an interesting summarization T. Coman reviews the data on the ultrastructure of respiratory organs in vertebrates and the evolutionary conclusions drawn from them.

The number contains several pathomorphological studies too, among others on morphological and histochemical changes observed under pathological conditions in the thyroid gland, cardiovascular system, skin and digestive tract. Special attention is called to a study by S. Constatinescu and G. Filipescu on mitochondria which is an exemplary work from the point of view of both its content and the wide range and up-to-dateness of the methods applied.

To sum up this necessarily brief and limited survey I should like to emphasize that owing to the great diversity of subjects, the valuable content and methodological up-to-dateness of investigations the periodical is highly noteworthy and can be recommended to every expert who deals with the problems of anatomy, histology and ultrastructural research.

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